

**Water Resources Research Institute of the
University of North Carolina
Annual Technical Report
FY 2017**

Introduction

During 2017-2018 (Fiscal Year 2017), the NC Water Resources Research Institute (WRRI) continued a program of excellence for fostering research, training and information dissemination efforts that are responsive to the water problems of the state and region. To develop its programs, the Institute maintains a proactive effort to interact and communicate with federal, state, and local water managers and other relevant stakeholders.

WRRI interacts closely with state agencies such as the NC Department of Environmental Quality, water and power utilities, and an array of research and outreach programs within the UNC system and at private higher educational institutions across North Carolina. The Institute utilizes an advisory committee that provides input, guidance, and review of the research priorities that are used in developing our Requests for Proposals (RFPs) and directing other research activities.

A technical review committee is also convened on an annual basis to advise WRRI staff on the scientific merit of research proposals submitted for funding. WRRI leverages USGS funds with a variety of sources such as the Urban Water Consortium, the Stormwater Group, and grants received by the Center of Excellence for Watershed Management.

WRRI team members are actively engaged in 15 boards and committees around the state where they bring expertise and perspective to efforts to address NC's water issues. These additional efforts help WRRI grow involvement in and support of water-related research and outreach across the state.

In response to the FY17 faculty RFP, WRRI received a total of 55 faculty pre-proposals from 11 institutions with a total request of \$5,683,984. Of these, 29 were invited to submit full proposals. WRRI ultimately selected 4 proposals to award, totaling \$400,000 (note that three of these proposals are 2-year awards and second year funding will be awarded through the following 104b cycle).

In response to the FY17 student RFP, WRRI received 12 proposals from 4 institutions with a total funding request of \$119,661. Five of these were selected for funding, totaling \$49,930. Funds for these projects come from USGS, state funds, and funds from two research consortia (the Urban Water Consortium and the Stormwater Group) administered by WRRI.

Projects resulting from the FY17 RFPs will be funded from March 1, 2018 to February 28, 2019 and details from those supported by USGS funds will be reported in the next USGS annual report.

From the FY16 student RFP issued jointly with NC Sea Grant, a NOAA federal-state program with coastal watershed and water quality goals that align with WRRI. Five new research projects totaling \$49,966 started during this reporting period. Of these, two were USGS projects totaling \$20,000. The other three projects, totaling \$29,966, were funded by NC Sea Grant.

Faculty projects active during this period were in year 2 of their 2-year awards issued from the FY15 RFP for FY16 funds. One additional faculty project, totaling \$39,599, was funded by the Stormwater Group.

Through the WRRI projects supported by only USGS funds in FY17, the Institute supported 22 students; researchers delivered 22 professional presentations about these projects; 3 peer-reviewed articles were published; and the WRRI-supported projects led the funded PIs to secure an additional \$1,279,674 in grant funding for continuation and expansion of the research projects (details available in the "Notable Awards and Accomplishments" section of this annual report).

In total, researchers with active projects during this reporting period who were funded through WRRI (including from USGS funds, state funds, and other consortia funds) supported 50 students, delivered 65 presentations, produced 8 publications, and successfully secured a total of \$1,370,625 in additional funding outside of WRRI for which their WRRI-supported project served as the foundation. This additional funding does not include the additional state match and private consortia funds that WRRI uses to match USGS funds and award additional research dollars.

The information transfer program continued to focus on disseminating results of sponsored research and providing information on emerging water issues, solutions, and regulations. Results of research are disseminated by publication of technical completion reports, peer reviewed manuscripts, summary posts on the WRRI website, and presentations by investigators at the WRRI Annual Conference and individual group meetings. Six internal research reports from WRRI projects were produced.

Through 14 education and training events, the WRRI's information dissemination program engaged a documented 1105 adult participants and 147 youth participants, though many events targeted additional unquantified audiences through webinars and public events. Additional stakeholders were reached through a number of meetings, focus groups, and other gatherings that are not captured in the official list of training events.

WRRI expanded the professional development credits it is able to offer to event participants. It continues to sponsor continuing education credits by the NC Board of Examiners of Engineers and Surveyors as an Approved Sponsor of Continuing Professional Competency activity for Professional Engineers and Surveyors licensed by the State of North Carolina. In addition, WRRI submits information for approval to the N.C. Board of Landscape Architects to offer contact hours to landscape architects. This year, WRRI was also approved to offer professional development credits for licensed geologists and for soil scientists, furthering both the value of our program to stakeholders and confirmation of the quality of WRRI programming.

Research Program Introduction

During 2017-2018 (Fiscal Year 2017), WRRI continued its focus of fostering research, training, and information transfer that is responsive to water issues of the state and region. Results from Institute-supported research efforts are expected to assist local, municipal, state, regional and federal agencies to improve their decision-making in the management and stewardship of their water resources.

To help it chart and sponsor a research program responsive to the water resource issues and opportunities in North Carolina, WRRI interacts closely with state agencies such as the NC Department of Environmental Quality, water and power utilities, and an array of research and outreach programs within the UNC system and at private higher educational institutions across North Carolina. The Institute utilizes an advisory committee that provides input, guidance, and review of the research priorities that are used in developing our Requests for Proposals (RFPs) and directing other research activities. This committee is composed 16 representatives of several federal and state agencies, local governments, industries, and non-governmental organizations (NGOs).

Based on in-depth discussions with stakeholders and advisory committee members regarding the most significant water research needs and priorities in NC, WRRI's research priorities are captured within four main RFP focus areas. Research priorities are incorporated into our Section 104b Objectives on an annual basis and included in our RFP. The RFP is sent to relevant offices of sponsored research at colleges and universities as well as an email distribution list of approximately 180 university faculty across North Carolina. WRRI also hosts informational webinars to orient potential applicants to WRRI, its research priorities, and the RFPs it releases. Full-time faculty members from all North Carolina institutions of higher education are eligible to receive grants from WRRI. Students RFPs are also issued and students receive funds through faculty sponsors.

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Faculty projects active during this period were in year 2 of their 2-year awards issued from the FY15 RFP. No new faculty awards from USGS funds were issued during this project period as FY16 funds had been pre-committed for the two-year projects.

One additional faculty project, totaling \$39,599, was funded by the Stormwater Group.

Research Program Introduction

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The role of environmental buffers in potable water reuse

Basic Information

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|---------------------------------|--|
| Title: | The role of environmental buffers in potable water reuse |
| Project Number: | 2016NC208G |
| USGS Grant Number: | |
| Start Date: | 9/1/2016 |
| End Date: | 8/31/2018 |
| Funding Source: | 104G |
| Congressional District: | NC-012 |
| Research Category: | Engineering |
| Focus Categories: | Wastewater, Water Quality, Water Supply |
| Descriptors: | None |
| Principal Investigators: | Olya Keen, Mariya Munir, Michael Meyers |

Publications

There are no publications.

The role of environmental buffers in potable water reuse

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WRRRI project number 2017-0356-01

May 12, 2017

1. Introduction

Estimated 14% of the US population lives in arid and semi-arid areas (US Census 2010). Those are also the areas experiencing the highest population growth according to the US Census data, with 5 arid states in the top 10 fastest growing states. Because of the insufficient water supplies to sustain the current and projected population, many of the arid areas practice or consider practicing potable water reuse. Apart from the acknowledged and purposefully implemented potable water reuse systems, there is a great number of instances where unacknowledged de facto potable water reuse is happening, i.e. when highly populated areas discharge treated effluent into the body of water that becomes a drinking water source for another downstream entity.

In most instances, water is released into an environmental buffer as it travels from a wastewater treatment plant (WWTP) to the downstream drinking water treatment plant (DWTP). Even in the instances of acknowledged water reuse, water is not pumped directly to the drinking water treatment plant but is rather allowed to percolate into an aquifer or to spend some time in a river or a reservoir. The reclaimed water that is destined for potable reuse applications is treated to the highest industry standards but subsequently is allowed to come in contact with various contaminants in the natural environment.

One of the main functions the environmental buffers serve is the improvement of public perception of water reuse, whether it is justified or not. The “yuck factor” is an important consideration in potable water reuse implementation projects (Schmidt 2008). Another potential benefit is environmental attenuation of contaminants via dilution, photolysis, hydrolysis, biodegradation and sorption. Some of the contaminants of concern in potable reuse water are pharmaceuticals and personal care products. While these contaminants are presently unregulated, multiple studies established their relevance to aquatic health. For example, chronic exposure to trace levels of pharmaceuticals have been demonstrated to cause disruption of predator avoidance patterns (Painter et al. 2009), feminization of male fish (Lange et al. 2008), and other endocrine disrupting effects (Connors et al. 2009). Apart from their relevance to environmental health, trace pharmaceuticals are linked to development of antibiotic resistance in the environment as a result of the contact between sub-inhibitory levels of antibiotics and microorganisms (Akiyama and Savin 2010, Goñi-Urriza et al. 2000). As a result, trace pharmaceuticals have direct relevance to human health. Other human health effects from chronic exposure to pharmaceutical mixtures in drinking water have been difficult to demonstrate and quantify, but are nevertheless possible (Pomati et al. 2006).

On the other hand, many of the contaminants, especially particulate matter and microorganisms can be reintroduced in the environmental buffer. As a result, the downstream DWTP requires treatment processes for removal of particulate matter and higher disinfectant levels which could be unnecessary if the DWTP were directly using treated wastewater effluent as source water. Environmental buffer can also introduce some of the unregulated emerging contaminants associated with urban and agricultural runoff, such as pesticides, herbicides and constituents of automotive fluids. In addition, trace levels of antibiotics discharged with treated wastewater get an opportunity to interact with microorganisms in the environment which could be one of the pathways of development of antibiotic resistance.

The main goal of this study is to answer the following questions: **Do environmental buffers mitigate contaminants or only public perception? Which contaminant classes get attenuated and which get introduced in the environmental buffer? How do specific types of environmental buffers (wetland, aquifer recharge, river, etc.) differ in that respect?** The

National Academy of Sciences assembled an expert panel on the water reuse topic in 2012, and one of the top seven research priorities identified by the panel for water reuse treatment efficiency and quality assurance was to develop a better understanding of contaminant attenuation in environmental buffers (National Academy of Sciences 2012).

The goal of this study is to measure the change in conventional water quality parameters as well as unregulated constituents of concern in several case studies representative of different types of environmental buffers. It will also estimate the costs to utilities for direct (pipe-to-pipe) vs. indirect (with environmental buffer) potable water reuse with each type of buffer involved.

If this study demonstrates that the environmental buffers serve only to recontaminate highly treated water and do little for attenuation of contaminants in most classes, the utilities armed with this information may ask an important question: Is it sensible to release highly treated water into the environment instead of taking it to the next level of engineered treatment? For example, water released into a river will require particle removal treatment at a DWTP. Instead, water that is already low in particulate matter coming from a WWTP could be treated by advanced treatment processes immediately. The cost saved on the particle removal and disinfection could be applied to advanced treatment processes which could remove trace contaminants in a controlled and therefore more efficient manner than an environmental buffer could. While the answer to this question may appear obvious, no study currently exists that addresses this question in a systematic manner.

This study will produce materials that utilities will be able to use to communicate to their customers on the topic of water reuse, environmental buffers, and associated water quality. Communication materials that are accessible to a layperson but provide information with sufficient level of scientific detail can improve public trust in policy making and can open an avenue for informed public feedback (Veldhuis 2015).

2. Project goals and objectives

The main goal of this study is to answer the following questions:

- Do environmental buffers mitigate contaminants or only public perception?
- Which contaminant classes get attenuated and which get introduced in the environmental buffer?
- How do specific types of environmental buffers (wetland, aquifer recharge, river, etc.) differ in that respect?

To answer these questions, the following objectives were proposed:

1. Evaluate the ability of different types of environmental buffers (groundwater recharge, riverbank filtration, wetland treatment, and discharge into a river and a lake) to attenuate contaminants representative of different classes and different environmental fate. Specifically, compare water quality of the WWTP effluent to the water quality at the influent to the DWTP after it has passed through the environmental buffer (McDowell WWTP and Franklin DWTP, NC; Denver Metro and Englewood/Littleton WWTP and Aurora Prairie Waters, CO; Orange County Water District recharge and production well water, CA). Determine which classes of contaminants get attenuated and which get reintroduced in each type of environmental buffer (wetland, aquifer recharge, alluvial flow, river and lake). This objective includes analysis of conventional contaminants (suspended

solids, microorganisms, etc.) along with emerging contaminants (pharmaceuticals and antibiotic resistance genes - ARG).

2. Estimate the cost of existing potable water reuse systems if no environmental buffer was used. Based on the results of Objective 1, develop recommendations for utilities for potable water reuse. The recommendations would include the discussion of the treatment technologies appropriate for potable water reuse and the necessity (or lack thereof) for environmental buffers. Evaluate the cost and the logistical possibility of implementing the suggested recommendations.
3. Develop public communication materials for utilities based on the findings. Prepare a public education document/module to promote the optimal potable water reuse scenario based on the research results. The research results will be adapted to lay audience.

3. Activities

3.1. Summary

The project commenced in September 2016. The first few months of the project were allocated to purchasing supplies, developing methods, student training and site visits. During this time, the graduate student travelled to the USGS Kansas laboratory for one week to be trained on extraction and analysis methods for emerging contaminants. The student worked to develop sampling protocols and establish logistics with the utilities. The PI Olya Keen travelled to one of the collaborating utilities (Orange County Water District) to identify the appropriate sampling locations. Meetings were also conducted with Charlotte Water for the same purpose. The third site for this project is less challenging logistically, and decisions were arranged via email and phone conversations.

The timeline in the proposal allocated the bulk of time to Objective 1 as the most time consuming. The original goal was to complete this task within 18 months from the commencement of the project. The project is currently 8 month completed with active sampling going on for 4 months. To-date, of the 18 planned sampling events, 5 sampling events have been fully executed and the 6th is in process. The sampling is on schedule to be completed by the proposed deadline of March 2018.

3.2. Sampling sites

To-date, samples have been analyzed from two of the three participating locations, each exhibiting different environmental buffers or a combination thereof used in either acknowledged or de facto water reuse.

Site 1: Orange County Water District (OCWD)

The site has groundwater recharge ponds that are supplied with water from two sources: (a) a constructed wetland that serves to purify river water (Santa Ana River) before it is routed to the groundwater recharge ponds; and (b) wastewater treatment plant effluent that went through advanced water purification system (AWPS) consisting of microfiltration, reverse osmosis and advanced oxidation. The water in Santa Ana River is largely impacted by effluent from two upstream wastewater treatment plants: San Bernardino and Riverside.

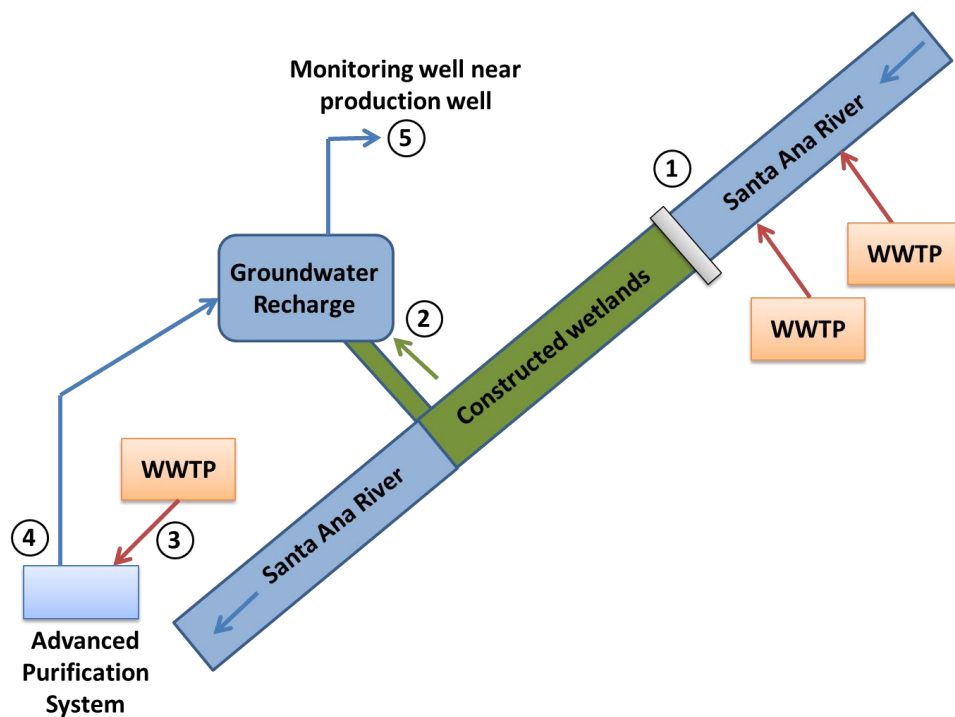


Figure 1: Sampling schematic for OCWD (It should be noted that a portion of Santa Ana River that is not used to feed the recharge basins bypasses the wetlands).

Site 2: Charlotte Water

Water from a local wastewater treatment facility flows with McDowell Creek into Catwaba River at the point where the river widens to form a lake (Mountain Island Lake). Water from Mountain Island Lake is used further downstream as a DWTP intake. Both Mountain Island Lake and Lake Norman located upstream are formed by dams on Catawba River and are located in highly urbanized areas.

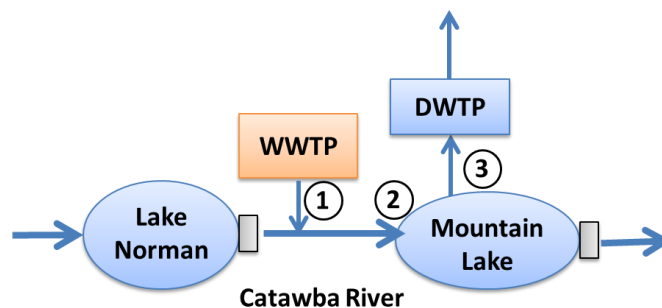


Figure 2: Schematics of Charlotte Water sampling locations

Site 3: Aurora Prairie Waters

The site uses South Platte River as a water source which largely consists of the effluent from several large wastewater treatment plants upstream: Englewood/Littleton and Denver Metro (two

collocated plants). The intake water is routed through a riverbank filtration system prior to advanced treatment that produces potable water delivered to utility customers.

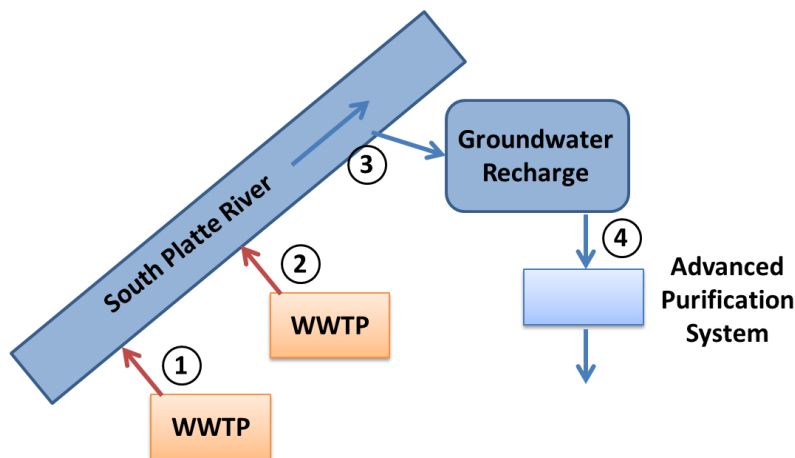


Figure 3: Sampling schematic for Aurora Prairie Waters

3.3. Sample collection, storage and processing

Participating utilities were supplied with coolers containing the description of the containers and the sampling technique. Containers were prepared per standard methods for each test and necessary preservatives were added as necessary (e.g. nitric acid for metals sampling). All samples were shipped with ice-packs to minimize exposure to heat, and maintained at 4 °C once they arrived at UNC Charlotte. Upon delivery, all sample containers were counted and verified according to the chain of custody forms. All broken or contaminated containers were recorded and valid substituting samples were used. Sample with 24 hour hold times were processed immediately, and remaining samples were processed before the corresponding hold time.

Biological samples (salmonella, coliform, E. coli, cryptosporidium, giardia and ARGs) are processed immediately. Salmonella, coliform, and E. coli enumeration is done using corresponding most probable number (MPN) methods and results can be obtained within 5 days. Cryptosporidium, giardia, and ARG enumeration results are obtained by using real-time polymerase chain reaction (qPCR). Samples are filtered using 0.45µm sterile nylon filters, vortexed in sterile phosphate dilution water (EPA Method 1680 2006), then centrifuged at 10,000rpm for 20min. The supernatant is then decanted and the concentrated filtered suspensions are currently stored at -80°C until further processing.

Metals and cations (B, Cd, Cu, Pb, Na, and Hg) are preserved in 2% nitric acid and stored in 4°C until further processing, currently scheduled to be analyzed on May, 10th 2017, using inductively coupled plasma optical emission spectrometry (ICP-OES). Anions (NO₃⁻, NO₂⁻, SO₄²⁻, PO₄³⁻, Cl⁻, Br⁻, and I⁻) are analyzed using ion chromatography (IC) and HACH test kits. Due to unforeseen column contamination, samples collected after March 13th for Cl⁻, Br⁻, SO₄²⁻, and I⁻ are still waiting to be analyzed. Replacement column is scheduled for delivery on May 15th.

Emerging contaminants (antibiotics: azithromycin, amoxicillin, cephalexin, ciprofloxacin, sulfamethoxazole-trimethoprim, doxycycline, levofloxacin, clindamycin, penicillin V; and

pharmaceuticals/pesticides: carbamazepine, sucralose, ibuprofen, glyphosate, and atrazine) will be analyzed using liquid chromatography-mass spectrometry and are extracted using solid phase extraction technique with hydrophilic-lipophilic-balanced (HLB) cartridges using the EPA method 1694 (Ferrer et al. 2010). Samples are eluted and stored at -20 °C in sterile glass vials. Samples are scheduled to be concentrated under nitrogen gas evaporation. Glyphosate is derivatized with HPLC grade 99% 9-fluorenylmethoxycarbonyl chloride (FMOC), and stored in 4°C waiting to be extracted (Lee et al. 2002). Benzo[a]pyrene will be analyzed using GC-FID following the EPA method 525.5.

Water characterization [total suspended solids (TSS), total organic carbon (TOC), pH, alkalinity, conductivity, 5 day biochemical oxygen demand (BOD₅), and chemical oxygen demand (COD)] are analyzed according to the EPA methods or Standard Methods (Rice et al. 2012). Nutrient analysis (total nitrogen and total phosphate) is done using HACH test kits. Results are all obtained prior to expiration of hold time.

Detailed methods and protocols are included in the Appendix 3, as well as a list of methods resources with links.

4. Findings and their significance

To-date, only conventional parameters have been analyzed on the collected samples. Samples for other parameters have been extracted and are preserved until more samples have been accumulated for a more efficient analysis.

Of the sites analyzed, the some trends can be remarked for various processes and are discussed in the sections below. These sections summarize and highlight the main observations with some of the more dramatic results shown in Figures 4 and 5. Tables containing results collected so far can be found in Appendix 4.

4.1. Attenuation in wetlands

Wetlands appear to be effective in decreasing the counts of wastewater indicator organisms (total coliforms, fecal coliforms, enterococci). Total coliforms were reduced by 94-99%, fecal coliforms by 91-95% and enterococci by > 98%. Salmonella counts did not show statistically significant change, but the counts in general were fairly low ranging from 0.7 to 8.8 MPN/100 mL. Wetland treatment also had a significant positive effect on lowering TSS by 96-97%, most likely due to slowing of the flow as the stream entered the wetlands, which allowed particulate matter to settle. Some of the reduction of microbial counts could be associated with the settling of particulates as well. The following parameters showed no observable change in wetland treatment based on the two samples collected to-date: TOC, BOD₅, conductivity, pH, alkalinity, and COD. There appears to be some incomplete denitrification as nitrite levels increase significantly (from 0.025 mg/L to 0.20 mg/L as N) while nitrate is lowered (from 4.5 mg/L to 1.1 mg/L as N). These values are averages of two samples. The increase in nitrite level is not high enough to cause a concern based on these observations (high value was 0.35 mg/L as N). Total nitrogen was lowered in wetland treatment by 30-70% while no significant decrease in total phosphorus was observed. The parameters that were negatively affected by wetland treatment were chloride and sulfate concentrations (increased from 20 mg/L to 148 mg/L and from 35

mg/L to 72 mg/L respectively). Anions were analyzed on one of the two samples, and it remains to be seen whether this trend is consistent.

4.2. Attenuation through advanced water purification

As expected, all parameters were majorly improved by advanced water purification. Microbial counts were all below detection level after treatment with very high levels in the influent (>2420 MPN/100 mL of total coliforms, fecal coliforms and enterococci, and 9-27 MPN/100 mL of Salmonella). TOC was reduced by 99% to 0.13 ± 0.01 mg/L, chloride was reduced by 98% to 4.5 mg/L, bromide by 79% to 0.02 mg/L, sulfate by 99% to 1.6 mg/L, BOD₅ by 98% to 0.3 mg/L to below detection limit, TSS by 92% to 0.2-0.8 mg/L, conductivity by 98% to 35 ± 9 mg/L, alkalinity by 96% to 5 ± 2 mg/L as CaCO₃, nitrate by 98% to 1.0 ± 0.0 mg/L as N, total phosphorus by 96% to 0.08 ± 0.03 mg/L, and COD by 90% to 4-15 mg/L. Results for samples with high consistency between the two sampling events are reported as an average with standard deviation margins, and the values showing larger range are shown as a range with the two measurements as the upper and lower end. pH lowered in the process from 7.2 ± 0.0 to 6.0 ± 0.4 . The water was collected prior to remineralization, and the pH of the finished water is raised closer to the influent pH after the sampling point. No iodide was detected in any samples, and nitrite was below detection limit (BDL) for all samples except one influent sample where nitrate was 0.215 mg/L as N. Phosphate was extremely low in the influent (0.02-0.04 mg/L) and was reduced further by approximately 70%.

4.3. Attenuation in groundwater recharge system

The groundwater recharge system is fed by recharge ponds that contain the water treated through wetlands and water from AWPS. Wetland treated water contained fairly high counts of bacteria. However, no tested organisms were detected in the monitoring well near the production well. The production well quality was close to that of AWPS in terms of TOC, Cl⁻, SO₄²⁻, BOD₅, TSS, conductivity. Slight increase in nitrate, bromide and alkalinity was observed. Alkalinity increase is a natural phenomenon and is most likely the result of dissolution of minerals during recharge. Nitrate and bromide, on the other hand, most likely come from non-point sources in the surrounding area and are the result of human activity. The concentrations of both remained very low and well below any levels of concern (1.5 mg/L as N for nitrate and 0.07 mg/L for Br⁻), although they demonstrate the potential for contamination of groundwater in aquifer recharge systems. Total phosphorus was the only parameter that was much higher in well water than in AWPS water, but still lower than in wetland effluent (Figure 4A). For comparison, chloride in the wetlands effluent, AWPS effluent and groundwater recharge monitoring well is also shown (Figure 4B) to indicate that the increase in total phosphorus cannot be attributed to the wetlands effluent and most likely enters groundwater from non-point sources. Phosphate remains low in all samples (wetland effluent, AWPS effluent and monitoring well), therefore the increase in total phosphorus can potentially be attributed to organic compounds containing phosphorus. While many of those are benign, samples will be analyzed for presence of organophosphate pesticides. Overall, groundwater recharge appeared to provide more benefit than risk based on the two samples and on the conventional parameters analyzed so far. It appears to provide a low-cost treatment to an impaired source (wetlands effluent) and it does not appear to significantly contaminate the highly purified reclaimed water.

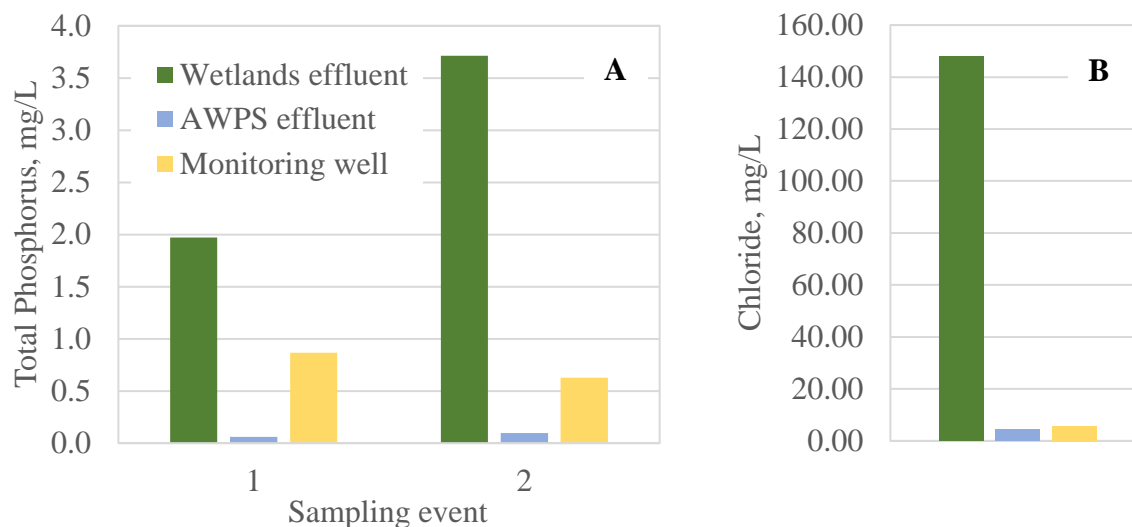


Figure 4: Total phosphorus (A) and chloride (B) concentration in groundwater recharge system monitoring well and in two sources used for groundwater recharge: wetlands-treated stream water consisting primarily of wastewater effluent and advanced water purification system (AWPS) effluent

4.4. Attenuation in a lake system

In general, lake water had good microbial quality with 1-3 MPN/100 mL of total coliforms, 0-3 MPN/mL of fecal coliforms, 0 MPN/100 mL of enterococci and 0.1-0.65 MPN/100 mL of *Salmonella* during the two dry weather sampling events. For the third sample that was collected after substantial rainfall, much higher levels of tested microorganisms were observed. Additionally, microorganism counts increased from the point where wastewater effluent mixed with the lake to the downstream point where drinking water intake is located (Figure 5). The increase in microorganisms is likely related to non-point sources (e.g. runoff that may contain animal excrement). Wastewater effluent microbial quality was not affected by increased rainfall, although the flow through the wastewater treatment plant and the need for temporary storage in equalization basins increased. To-date, only one wet weather sample of the three planned was collected, and future data will reveal whether the observations are trends for wet weather. The lake system in general appears to provide a substantial dilution for a number of parameters: TOC, alkalinity, conductivity, nitrate and COD. TSS was comparable in effluent and in lake water. Nitrite was below detection limit for all samples. There was some attenuation of total nitrogen and total phosphorus in the lake system.

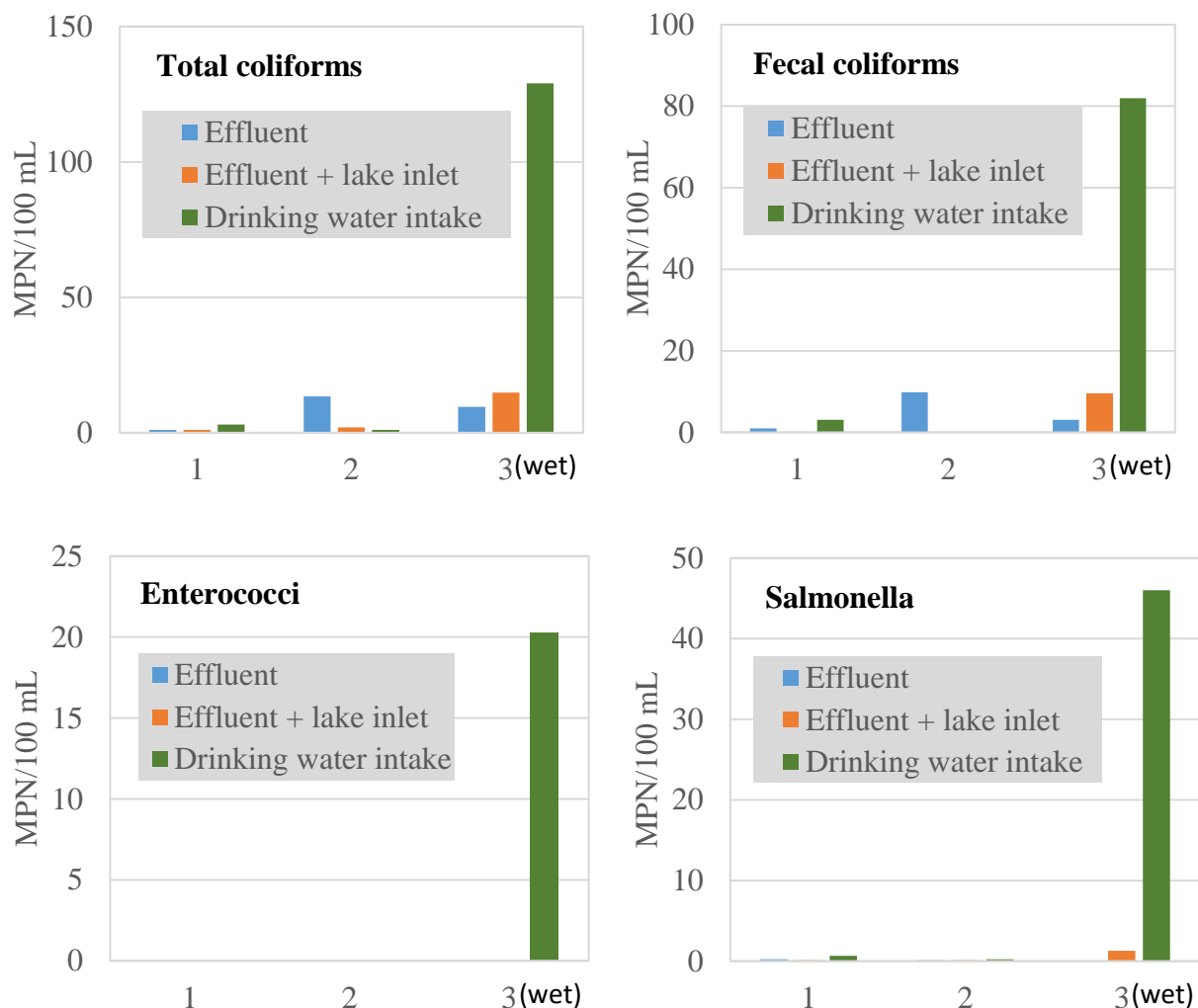


Figure 5: Effect of wet weather on microbial quality of the lake environmental buffer. Microbial counts for three sampling events, one of which was impacted by wet weather.

4.5. Next steps

Sampling is currently underway at the third location in this study: river attenuation and riverbank filtration site. Sampling is scheduled to go on through March of 2018 and is currently ~30% complete. In September-October of 2017, it is anticipated that enough data will be collected to begin an economic and regulatory analysis of the use of environmental buffers vs. direct potable reuse. In August-September, the first batch of extracted samples will be analyzed on HPLC-MS and GC for organic contaminants of interest to this study. In February-March, as sampling and analysis nears the end, participating utilities will be contacted regarding developing a public communication message/module to disseminate the results of the study.

4.6. Significance of findings

Although the original hypothesis was that the environmental buffers mainly serve to mitigate the public perception of water reuse, limited data collected so far suggest that at least from the perspective of conventional water parameters, environmental buffers can be of value or at least

of no harm. Currently it appears that wetlands can be effective for mitigating microbial contamination and TSS of an otherwise impaired water source with influent microbial counts of ≥ 550 MPN/100 mL and TSS of up to 293 mg/L, and can provide some marginal reduction in total nitrogen. It must be noted that both of the wetland samples analyzed to-date are wet weather samples, and it is possible that the microbial contaminants and the TSS levels in the influent are lower in dry weather and that the effect of the wetlands on improving those parameters is much less pronounced. Groundwater recharge system was highly effective in removing microbial contaminants, TSS, TOC and in general had levels of all measured conventional parameters close to those of highly purified water. The lake system in this study provided an effective attenuation by dilution, with many conventional water quality parameters in the lake water better than in treated wastewater effluent. However, it wet weather when runoff volumes were high, the lake was susceptible to microbial contamination that was not observed in dry weather.

5. Student involvement

The project involves a graduate and an undergraduate student. The graduate student Xueying Wang has worked to develop the methods, the sampling schedule, has been coordinating with the sampling location contacts and handling the more complex analysis: microbial testes, sample extractions, tests requiring the use of complex instruments, e.g. IC, ICP-OES, GC and HPLC/MS. She has travelled to the USGS laboratory in Kansas to be trained on trace organics extraction and analysis. Additionally, an abstract was submitted to AWWA International Symposium on Potable Reuse, which will take place January 22-23, 2018 in Austin, TX. The presentation will be given by Xueying who is the first author on the abstract.

The undergraduate student, Brittany Hause, has worked under Xueying's supervision. Her tasks are to measure routine water quality parameters (BOD₅, TSS, pH, alkalinity, etc.) including all relevant QA/QC.

6. Deviations from original project plans

Any changes to the project are minor and are not expected to affect the ability to address the project objectives. Challenges encountered so far are below:

- a. Sampling of the Charlotte location where the effluent from the wastewater treatment plant mixes with the lake inlet turned out to be inaccessible from the shore due to a steep drop at the available non-private-property location that was previous considered. A canoe was purchased, and the students travel approximately 1.5 mi by canoe to access the intended location from water.
- b. The Denver Metro wastewater treatment plant consists of two separate plants, and the operators did not consider it possible to collect a mixed sample based on the sampling protocols. Therefore, two separate samples will be collected from that location and analyzed, rather than one mixed sample as originally planned. It currently appears that the budget is sufficient to accommodate this additional sampling location.

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Appendix 1: Abbreviations and symbols

Abbreviations:

ARG = antibiotic resistance genes
AWPS = advanced water purification system
BDL = below detection limit
BOD₅ = 5-day biochemical oxygen demand
COD = chemical oxygen demand
DWTP = drinking water treatment plant
FMOC = 9-fluorenylmethoxycarbonyl chloride
GC = gas chromatography
GC-FID = gas chromatography - flame ionization detector
HLB = hydrophilic-lipophilic balanced
HPLC = high performance liquid chromatography
HPLC-MS = high performance liquid chromatography - mass spectrometry
IC = ion chromatography
ICP-OES = inductively coupled plasma - optical emission spectrometry
MPN = most probable number
OCWD = Orange County Water District
QA/QC = quality assurance/quality control
qPCR = real-time (quantitative) polymerase chain reaction
TOC = total organic carbon
TSS = total suspended solids
WWTP = wastewater treatment plant

Symbols:

B = boron
Br⁻ = bromide
CaCO₃ = calcium carbonate
Cd = cadmium
Cl⁻ = chloride
Cu = copper
Hg = mercury
I⁻ = iodide
N = nitrogen
Na = sodium
NO₂⁻ = nitrite
NO₃⁻ = nitrate
Pb = lead
PO₄³⁻ = phosphate
SO₄²⁻ = sulfate

Appendix 2: Results dissemination, research products

As the project is currently only approximately 30% complete, the results collected are not ready for dissemination. An abstract was submitted in April to AWWA International Symposium on Potable Reuse, which will take place January 22-23, 2018 in Austin, TX. Much of the data is expected to be collected and processed by January. After the conference, the research team will work on publishing the results.

Appendix 3: Analytical protocols

Microbiology

Salmonella

Membrane Filtration for Salmonella Concentration (9260B-1d)

1. Low turbidity water – Filter several liters (note the amount used) through a sterile 142-mm (0.45 µm) membrane filter

High turbidity water – Precoat sterile 142-mm (0.45 µm) membrane filter with 500 mL of diatomaceous earth suspension (aids filtration).

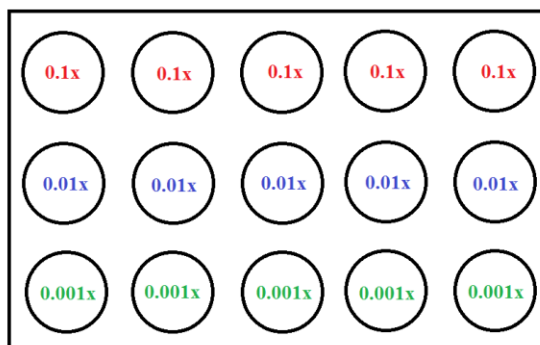
2. Immediately add desired sample water volume to the filter without interrupting filtration.
3. Place filtered membrane in a sterile blender jar containing 100 mL sterile peptone water and homogenize at high speed for 1 min.

Microbial Enrichment for MPN Method (9260D)

4. Make serial dilution of the sample homogenate with double strength selenite cysteine broth (0.1x, 0.01x, and 0.001x) total volume 50 mL.

| Dilution | Concentration of stock sample homogenate/selenite cysteine broth (mL/100 mL) | Volume of stock homogenate added (mL) | Volume of selenite cysteine broth added (mL) | Final concentration of sample homogenate to selenite cysteine broth (mL/L) |
|----------|--|---------------------------------------|--|--|
| 0.1x | n/a | 2.5 mL | 22.5 mL | 0.1 |
| 0.01x | 10/100 | 2.5 mL | 22.5 mL | 0.01 |
| 0.001x | 1/100 | 2.5 mL | 22.5 mL | 0.001 |

5. Proportion sample homogenate into a five-tube, three-dilution multiple-tube procedure in double strength selenite cysteine broth (9221C and 9260B 2-a). Perform in 50 mL centrifuge vials.



6. Incubate MPN glass vials for 48 h at 35-37 °C (time and temperature specific to selenite cysteine broth enrichment).
7. After incubation, using sterile inoculation loop, streak from each MPN vial to individual plates of brilliant green and xylose lysine desoxycholate agars. Incubate upside down (to prevent condensation from falling onto the plates) for 24 hours at 35°C

8. Select from plate at least 1 (preferably 2-3) salmonella colonies, using sterile cell scraper, inoculate a triple sugar iron and lysine iron agar slant tube*. Look for white or opaque black bacterial colonies.
 - a. Sterilize the inoculating needle in the blue flame of the Bunsen burner till red hot and then allowed to cool.
 - b. Take a sterile TSI or LIA slant tube from the rack, remove the cap and flame the neck of the tube.
 - c. Stab the needle containing the pure culture into the medium, upto the butt of the TSI/LIA tube, and then streak the needle back and forth along the surface of the slant.
 - d. Again flame the neck of the TSI/LIA tube, cap it and place it in the test tube rack.
 - i. TSI slant (Salmonella produces alkaline red slants and acid yellow butt with/without gas bubbles, and blackening). Salmonella is a non lactose fermenter thus have pink slant and yellow butt
 - ii. LIA slant (salmonella produces black butt with red slant)
9. Estimate Bacterial Density using most probable number (MPN) (EPA Table 9221:IV and 9221C)

Total and Fecal Coliforms and Enterococci

Total coliform and E. coli (fecal coliform)

The method Colilert-18/Quanti-Tray or Quanti-Tray/2000 for water analysis is granted NF Validation by AFNOR Certification as an alternative method to the standard ISO 9308-3 for enumeration of Escherichia coli β -glucuronidase positive in bathing water, under the Certificate number: IDX 33/02-06/12.

Quanti-Tray Enumeration Procedure (including Absence/Presence)

1. Place 100mL of sample in a sterile mL IDEXX vessel (with sodium thiosulfate).
2. Add the contents of one pack of colilert reagent to the vessel.
3. Cap vessel and shake until thoroughly dissolved.
4. Pour sample/reagent mix into a Quanti-Tray/2000
5. Seal the tray with the IDEXX Quanti-Tray Sealer.
6. Place the sealed tray in a 35 ± 5 °C incubator for 18-22 hours (at the researcher's convenience).
7. Sample observation conditions and interpretation using Table 1. below
 - a. Compare incubated samples to the comparator in normal lighting conditions for total coliforms
 - b. Compare sample under 6 watt 365 nm UV light in darkened environment with comparator for E. coli.
8. Using tables provided by IDEXX obtain the most probable number for total coliform and E. coli.

Quality Control/Quality Assessment

1. Repeat above steps with 100 mL of sterile ultrapure water
2. Repeat above steps with active E. coli cultures

Table A3-1. Results interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

| Appearance of Vessel | Result |
|---|---|
| Less yellow than the comparator ¹ when incubated at 35 ± 0.5 °C or 44.5 ± 0.2 °C | Negative for total coliforms and E. coli; Negative for fecal coliforms |
| Yellow equal to or greater than the comparator when incubated at 35 ± 0.5 °C | Positive for total coliforms |
| Yellow equal to or greater than the comparator when incubated at 44.5 ± 0.2 °C | Positive for fecal coliforms |
| Yellow and fluorescence equal to or greater than the comparator when incubated at 35 ± 0.5 °C | Positive for E. coli |

Total Enterococci

Enterolert detects enterococci, such as E. faecium and E. faecalis, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology (DST). When enterococci utilize their β -glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl β -D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 CFU per 100 mL sample within 24 hours.

Quanti-Tray Enumeration Procedure (including Absence/Presence)

1. Place 100 mL of sample in a sterile IDEXX vessel (with sodium thiosulfate).
2. Add the contents of one pack of enterolert reagent to the vessel.
3. Cap vessel and shake until thoroughly dissolved.
4. Pour sample/reagent mix into a Quanti-Tray/2000
5. Seal the tray with the IDEXX Quanti-Tray Sealer.
6. Place the sealed tray in a 41 ± 0.5 °C incubator for 24 hours
7. Sample observation conditions and interpretation using Table 2
 - a. Observe sample under 6 watt 365nm UV light in darkened environment to check for fluorescence.
8. Using tables provided by IDEXX obtain the most probable number for enterococci

Quality Control/Quality Assessment

1. Repeat above steps with 100 mL of sterile ultrapure water
2. Repeat above steps with active E. faecalis cultures

Table A3-2. Results interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

| Appearance of Vessel | Result |
|----------------------|--------------------------|
| Lack of fluorescence | Negative for enterococci |
| Blue fluorescence | Positive for enterococci |

Real-time polymerase chain reaction (qPCR)

PCR sample filtration/concentration for cryptosporidium/giardia and antibiotic resistant genes

Filtration Method

Blank

1. Set up sterile filter apparatus
 - a. Sterilize forceps with flame
 - b. Using forceps, place sterile membrane filter on mesh lined side up
2. Filter 1 L of 18 M Ω – ultrapure water (or note volume used)
3. Place filter in the 50 mL centrifuge tube containing 30 mL of buffer solution.
 - a. Sterilize forceps and gently pick up the used filter.
 - b. Carefully roll the filter (lined side in) and place it in the centrifuge tube.
 - c. Take care not to cross contaminate.

Sample

1. Use the same filter apparatus as the blank (without re-sterilization)
 - a. Sterilize forceps with flame
 - b. Using forceps, place sterile membrane filter on mesh lined side up
2. Filter sample (note volume used)
 - a. If sample is turbid, more than one filter can be used.
3. Place filter in the 50 mL centrifuge tube containing 30 mL of buffer solution.
 - a. Sterilize forceps and gently pick up the used filter.
 - b. Carefully roll the filter (lined side in) and place it in the centrifuge tube.
 - c. Take care not to cross contaminate.

Concentration Method

1. Vortex centrifuge tube containing the filter for a minimum of 2 minutes.
 - a. Vortex in short intervals as careful not to tear the filters.
2. Remove filter using sterilized forceps
 - a. If pieces break off, remove all pieces with sterilized inoculation loop
3. Centrifuge the solution
 - a. 10000 rpm for 20 minutes
 - b. rotate the tube and centrifuge again for 3 minutes at 5000 rpm
4. Decant the supernatant with a sterile pipette until 4mL of the solution remains
 - a. DO NOT DISTURBE THE PELLET COLLETED AT THE BOTTOM
5. Mix the pellet with the remaining 4mL of buffer solution using a flamed-sterile loop until completely dissolved/homogenized.
6. Transfer the homogenized concentrate to a cryogenic tube using a sterile pipette and store at -80°C.

Nutrients

Chemical Oxygen Demand (HACH/EPA Method 8000)

1. Homogenize samples by shaking the sample container for 30 seconds.
2. Set the DRB200 Reactor power to on. Preheat to 150 °C.
3. Remove the cap from a vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of sample to the vial.
4. Remove the cap from a second vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of deionized/ultrapure water to the vial.
5. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.
6. Hold the vials by the cap, over a sink. Invert gently several times to mix.
7. Put the vials in the preheated DRB200 reactor. Close the lid and heat the vials for 2 hours.
8. Set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less.
9. Invert each vial several times while it is still warm.
10. Put the vials in a tube rack to cool to room temperature.
11. Start program 431 COD ULR, 430 COD LR or 435 COD HR.
12. Clean the blank sample cell.
13. Insert the blank into the cell holder. Push ZERO. The display shows 0 or 0.0 mg/L COD. Clean the prepared sample cell. Insert the prepared sample into the cell holder.
14. Push READ. Results show in mg/L COD.

Total Nitrogen (HACH/EPA Method 10072)

1. Start the DRB200 reactor. Set the temperature to 105 °C.
2. Use a funnel to add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials. Make sure to clean any reagent that gets on the lip of the vials or on the vial threads.
3. Add 0.5 mL of sample to one of the vials.
4. Add 0.5 mL of ultrapure water to the second vial.
5. Put the caps on both vials. Shake vigorously for at least 30 seconds to mix. Undissolved powder will not affect the accuracy of the test.
6. Put the vials in the reactor and close the lid. Leave the vials in the reactor for exactly 30 minutes.
7. At 30 minutes, use finger cots to immediately remove the vials from the reactor. Let the vials cool to room temperature.
8. Start program 394 N, Total HR TNT.
9. Add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.
10. Put the caps on both vials. Shake for 30 seconds.
11. Start the instrument timer. A 3-minute reaction time starts.
12. After the timer expires, remove the caps from the vials. Add one TN Reagent B Powder Pillow to each vial.

13. Put the caps on both vials. Shake vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect the accuracy of the test. The solution will start to turn yellow.
14. Start the instrument timer. A 2-minute reaction time starts.
15. When the timer expires, use a pipet to put 2 mL of the digested, treated prepared sample/blank into one TN Reagent C vial.
16. Put the caps on both vials. Invert 10 times to mix. Use slow, deliberation inversions for complete recovery. The vials will be warm to the touch.
17. Start the instrument timer. A 5-minute. Reaction time starts. The yellow color will intensify.
18. When the timer expires, clean the blank vial.
19. Insert the blank vial into the 16-mm cell holder.
20. Push ZERO. The display shows 0 mg/L N. Clean the sample vial. Insert the sample vial into the 16-mm cell holder.
21. Push READ. Results show in mg/L N.

Total Phosphorus (HACH/EPA Method 8190)

1. Start the DRB200 Reactor. Preheat to 150 °C.
2. Start program 536 P Total/AH PV TNT.
3. Add 5.0 mL of sample to the Total Phosphorus Test Vial.
4. Add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.
5. Put the cap on the vial. Shake to dissolve the powder. Insert the vial into the reactor. Close the reactor.
6. Start the instrument timer. A 30-minute reaction time starts.
7. When the timer expires, carefully remove the vial from the reactor. Set the vial in a test tube rack. Let the vial cool to room temperature.
8. Add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial.
9. Put the cap on the vial. Invert to mix. Clean the vial. Insert the vial into the 16-mm cell holder. Push ZERO. The display shows 0.00 mg/L PO_4^{3-} .
10. Add the contents of one PhosVer 3 Powder Pillow to the vial.
11. Put the cap on the vial. Shake to mix for 20–30 seconds. The powder will not dissolve completely. Start the instrument timer. A 2-minute reaction time starts. Measure the sample within two to eight minutes after the timer expires.
12. Clean the vial. Insert the vial into the 16-mm cell holder. Push READ. Results show in mg/L PO_4^{3-} .

5-Day Biochemical Oxygen Demand (EPA Method 5210 B)

Preliminary Work

1. Autoclave all glass BOD bottles and stoppers.
2. Prepare dilution water
 - a. Obtain appropriate volume of DI water in polypropylene container(s) 2 days prior to sample analysis.
 - b. Autoclave the DI water.

- c. Add buffer nutrients immediately prior to sample analysis to prevent unwanted microbial growth.
- d. Prior to sample dilution
 - i. Shake the dilution water container prior to sample analysis to ensure dissolved oxygen saturation.
 - ii. Check to ensure the dilution water DO level is at least 7.5 mg/L.
 - iii. If DO level is less than 7.5 mg/L, continue to aerate and shake container until the desired DO level is reached.
3. Calibrate DO meter per instructions on the back.

Sample Analysis

1. Adjust sample temperature to $20 \pm 3^{\circ}\text{C}$
2. Check sample pH
 - a. If pH is not between 6.0-8.0, adjust pH to 7.0 – 7.2 using sulfuric acid or sodium hydroxide.
3. Make twin dilution bottles

3 dilutions bottles and one quality control

| Bottle # | Dilution water volume (mL) | Sample water volume (mL) |
|----------|----------------------------|--------------------------|
| 1, 4 | 50 | 250 |
| 2, 5 | 150 | 150 |
| 3, 6 | 200 | 100 |
| 4*, 7* | 300 | 0 |

*Quality control

4. After dilution, measure the DO of one set of twin bottles and record it.
5. Stopper and parafilm the other set of twin bottles
 - a. Place bottles in a dark environment to prevent photosynthetic growth
 - b. Incubate bottles at temperature between $20 \pm 3^{\circ}\text{C}$
6. After 5 days, measure the DO of the incubated bottles and calculate BOD using the formula:

$$\text{BOD}_5 \text{ (mg/L)} = \frac{(\text{Initial DO} - \text{Final DO})}{\left(\frac{\text{mL of sample}}{300 \text{ mL}}\right)}$$

QA/QC

1. Sample bottles should have a minimum DO depletion of 2.0 mg/L and a residual DO of 1.0 mg/L
2. The control dilution water should not have a DO depletion of more than 0.20 mg/L

Anions

Total Phosphate (HACH/EPA Method 8048)

1. Start program 535 P React. PV TNT.
2. Add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Vial. Put the cap on the vial. Invert to mix.
3. Clean the vial. Insert the vial into the 16-mm cell holder. Push ZERO. The display shows 0.00 mg/L PO_4^{3-} .
4. Add the contents of one PhosVer 3 Phosphate Powder Pillow. Put the cap on the vial. Shake for at least 20 seconds. The powder will not dissolve completely.
5. Start the instrument timer. A 2-minute reaction time starts. When the timer expires, clean the vial.
6. Insert the vial into the 16-mm cell holder. Push READ. Results show in mg/L PO_4^{3-} .

Total Nitrate (HACH/EPA Method 10206)

1. Use a pipet to add 1.0 mL of sample/blank to the test vial. Use a pipet to add 0.2 mL of Solution A to the test vial.
2. Tighten the cap on the vial and invert until completely mixed.
3. Start the reaction time of 15 minutes. When the timer expires, clean the vial.
4. Using DR 1900 only: Select program 835.
5. Insert the vial into the cell holder. DR 1900 only: Push READ. Results show in mg/L NO_3^- -N.

Total Nitrite (HACH/EPA Method 10237)

1. Carefully remove the lid from the DosiCap™ Zip cap. Remove the cap from the test vial.
2. Use a pipet to add 0.2mL of sample to the test vial. Immediately continue to the next step.
3. Turn the DosiCap Zip over the test vial so that the reagent side goes on the vial. Tighten the cap on the vial.
4. Shake the vial 2–3 times to dissolve the reagent in the cap. Look through the open end of the DosiCap to make sure that the reagent has dissolved.
5. Start the reaction time of 10 minutes. When the timer expires, clean the vial. DR 1900 only: Select program 840.
6. Insert the vial into the cell holder. DR 1900 only: Push READ. Results show in mg/L NO_2^- -N.

Sulfate, chloride, iodide, bromide (Ion Chromatography)

1. Filter 40 mL of sample through 0.45 μm glass fiber filter.
2. Transfer sample to 10 mL IC sample vials.
3. Run sequence for EVERY SITE (even if multiple sites are run within the same day):
 - a. 1 blank (ultrapure water)
 - b. 1 standard (25 mg/L (I^- , Cl^- , SO_4^{2-}) and 2.5 mg/L (Br^-)) at the beginning and every 10 samples.
 - i. 2 mL of 100 mg/L stock + 6mL of ultrapure water = 25 mg/L (8 mL total)

- c. Spike samples for every sample site (3 samples + 1 sample with spike, 4 samples per site total).
 - i. Added spike concentration 12.5 mg/L
 - ii. Add 0.1 mL of 1000 mg/L stock (0.1mL from each original stock solution, (I⁻, Cl⁻, and SO₄²⁻) and 0.1 mL of 100 mg/L Br⁻ stock to 7.6 mL of sample water (8mL total)

(Blank – Standard – Sample 1 – Sample 2 – Sample 3 – Spiked Sample)

Eluent solution

0.9539 g Na₂CO₃ + 0.2352 g NaHCO₃ + 2 L ultrapure water

Metals

Inductively coupled plasma atomic emission spectroscopy

Boron, copper, sodium, lead, cadmium, and mercury

Sample Preparation and Storage

1. Samples are collected in metal free, nitric acid rinsed polypropylene plastic bottles.
2. Samples are preserved in nitric acid and stored in 4 °C until analysis.

Emerging Contaminants

Liquid Chromatography/Mass Spectrometry

Pesticides, pharmaceuticals, and antibiotics

Preliminary Work

1. Obtain 1 mL sample weight.
 - a. Weigh empty glass vial and record weight (W1).
 - b. Add 50/50 1mL methanol and acetonitrile and weight test tube, record the weight (W2).
 - c. (W2) – (W1) = weight of 1 mL of solution.
 - d. Or use tare for the weight of 1mL solution
2. Adjust pH to ≤ 2
3. Filter sample through 0.2-0.8 μ m glass fiber filter.
4. Rinse all glassware (including glass test tube) 3 times with HPLC grade water.
5. Add deuterated carbamazepine-D₁₀ to sample water for loss recovery.
 - a. Stock solution = 1 μ g/mL
 - b. Per 1 L of filtered sample, add 1mL of the 1 μ g/mL solution.
 - i. 1 μ g of carbamazepine-D₁₀ per liter of sample.

Solid Phase Extraction

1. Wash the HLB cartridge with 5 mL of methanol.
2. Condition the cartridge with 5 mL of solvent grade water.
3. Elute the sample through the cartridge.
 - a. Note the start and end time for rate calculation.
4. Elute the sample cartridge with 2.5 mL methanol then 2.5 mL acetonitrile.

5. Evaporate the test tube using nitrogen evaporator until < 1mL remains
6. Using the weighing method, reconstitute the sample using 50/50 methanol and acetonitrile until the weigh reaches previously recorded for the 1 mL solution.
7. Transfer sample to HPLC vial and store in -20 °C indefinitely.

Glyphosate

Sample Preparation

Sample should be derivatized within 5 days and stored in 4 °C in dark

1. Filter sample water
2. Dispense 10 mL of sample into plastic vials.
3. Add 200 µL of the 50 µg/L working standard to each of the 10 mL samples (per 10 mL sample will have the concentration of 1 µg/L of the isotope)
 - a. Do QA/QC every 10 samples (SPE per batch so add internal standard to batch)
 - b. Internal standard calculation
 - i. Internal standard stock made at 100 µg/L
 - ii. Add 100 µL to make up to 10 mL of sample
0.1 mL of 100 µg/L glyphosate-D₃ stock + 9.9 mL of sample
4. Add 0.5mL of 5% sodium borate solution.
5. Mix the solutions in the tubes (vortex is recommended).
6. Add 1.5 mL of 2.5 mM FMOC in acetonitrile.
7. Invert 3 times to mix
8. Place all tubes in 40 °C water bath in the dark for incubation for 24 ± 1 hours.
9. After incubation add 0.6 mL of 2% phosphoric acid in HPLC grade ultrapure water to the tubes.
10. Inver 3 times to mix
11. Store derivatized samples in the dark at 4 °C until analysis.

Solid Phase Extraction

1. Condition HLB cartridge sequentially with:
 - a. 2 mL of methanol
 - b. 2 mL of DI water
2. Load the 10 mL of derivatized samples.
3. Wash cartridge with 1 mL of DI water
4. Elute with 5 mL of 50/50 ammonia acetate in HPLC grade water and ACN
5. Evaporate until 1mL of below 1mL reconstitute with 50/50 mixture.
6. Store elution in -20 °C indefinitely.

Gas Chromatography (EPA Method 6440B-3)

Benzo[a]pyrene

Sample Collection and Storage (EPA-Method 525.2-8.0)

1. Sample Collection
 - a. Collect samples in 1L (Teflon lined cap screws) ashed amber glass bottles
 - b. Sampling equipment must be free of plastic tubing, gaskets and other parts that may leach interfering analytes into water sample.
 - c. Add 40-50 mg of sodium sulfate to each sample, stir/shake until dissolved (to reduce chlorine).
 - d. Add 6 N HCl to sample until pH is < 2 (to reduce microbial degradation of analyte)
 - e. Keep container sealed until extraction/analysis
2. Sample Preservation and Hold Time
 - a. All samples are iced/refrigerated at 4 °C away from light
 - b. Samples must be extracted within 14 days (stored at 4 °C away from light)
 - c. Extracts must be analyzed within 30 days after extraction.

Solid Phase Extraction

Preliminary Work

- 1) Wash all glassware in dish washer then rinse 3 times with ultrapure water.
- 2) Obtain glass tube and mark 6 mL and 10 mL volume line.
- 3) SPE cartridge: Bond-Elut 500 mg

Extraction Method

- 1) Filter 1000 mL of sample water through 0.45 µm filter.
- 2) Add internal standard for loss recovery (benzo[a]pyrene) to sample water.
- 3) Condition the cartridge sequentially with:
 - a. 4 mL ethyl acetate
 - b. 4 mL dichloromethane
 - c. 4 mL methanol
 - d. 4 mL water
- 4) Load sample into conditioned cartridge
- 5) Air dry cartridge for 30 minutes.
- 6) Elute cartridge sequentially into marked glass vial with:
 - a. 4 mL ethyl acetate
 - b. 4 mL dichloromethane
- 7) Add (1:1) ethyl acetate/dichloromethane solution to glass vial until volume reaches 10 mL.
- 8) Air evaporate 4 mL of the solution.
- 9) Transfer into in clean GC vial.
- 10) Store indefinitely in 4 °C away from light.

Gas Chromatography Method

Accessories

1. Detector, flame ionization (FID)

2. Column, Rxi-17Sil, MS (15 m-long x 0.25 mm-internal diameter, 0.25 μ m) (cat.# 14120)
3. Liner, 4 mm Split Precision Liner with glass wool (cat.# 21022)
4. Instrument, Shimadzu GC 2014

GC Operation Conditions (6440B-3c)

1. Sample
 1. Diluent: methylene chloride (care to avoid evaporation)
 2. Concentration: 20 ng/ μ L
2. Injection
 1. Volume: 1 μ L split (split ration 20:1)
 2. Temperature: 275 $^{\circ}$ C
 3. Split vent flow rate: 42 mL/min
3. Oven
 1. Temperature: 80 $^{\circ}$ C (hold 1 min.) to 320 $^{\circ}$ C at 15 $^{\circ}$ C/min (hold 2 min.)
4. Carrier Gas
 1. Helium (He)
 2. Constant flow at 2 mL/min.
5. Detector
 1. Temperature: 340 $^{\circ}$ C
 2. Constant column + constant make-up: 50 mL/min.
 3. Gas type: Nitrogen (N₂)
 4. Data rate: 20 Hz

Quality Control/Quality Assessment (EPA Method 525.2-9.3, modified)

QA/QC performed at the beginning of each sample batch run and after every 20 samples

1. 4 replicas of analyte concentration in the middle of the calibration range.
 - a. Add the appropriate aliquot of HCl and sodium sulfite to each analyte to standardize field and lab samples.
 - b. For each analyte replica, the mean accuracy, expressed as a percentage of the true value should be between 70-130%.
 - c. The relative standard deviation should be < 30%
2. Internal standard recovery should be > 70%
3. Laboratory fortified blank should be below the method detection limit
 - a. Utilizing Method Detection Limit Calculator by the EPA

Water Characterization

Total Suspended Solids

Preliminary Work

1. Prepare the filters
 - a. Hold the filters using cleaned forceps/tweezers.
 - b. Drag the filter back and forth in ultrapure water ≈ 3 times, or until filter no longer gives off white residue.
 - c. Place filters on a clean metal pan or aluminum sheet and dry in the oven at 120°C for 1 hour or until filter is completely dried.
 - d. Immediately place filters in desiccator directly from the oven until cooled.

Sample Analysis

1. Place the cleaned filter on a piece of aluminum.
 - a. Write the sample information on the aluminum with sharpie.
2. Weigh the clean filter and the aluminum and record the weight (W1)
3. Filter 100 mL of sample through the $0.45\ \mu\text{m}$ filter.
 - e. Handle the filter using only forceps/tweezers
4. Place filter on the corresponding aluminum foil and dry in oven at $103\text{-}105^{\circ}\text{C}$ for at least 1 hour.
5. Once dried, immediately place the filters in desiccator directly from the oven until cooled.
6. Weight the dried filter and aluminum foil (W2)
7. Follow the formula below for calculating TSS:

$$TSS(g/L) = \frac{W2(g) - W1(g)}{mL\ of\ sample} * 1000$$

Conductivity

1. Submerge conductivity probe in sample
2. Wait until readout is stable
3. Record results in μS units

pH

1. Check probe accuracy
 - a. Compare readout to standardized pH solutions on the counter (colorized)
 - b. Calibrate if not within ± 0.25 units
2. Submerge pH probe in sample
3. Wait until readout is stable
4. Record results

Total Organic Carbon

Preliminary Work

1. Prepare 1 g-carbon/L stock organic carbon standards using potassium hydrogen phthalate (KHP)

- a. Weigh 1.0 g of KHP and dry in oven at 103-105 °C for 2-3 hours.
 - i. Place immediately in desiccator directly from oven until cooled
 - b. Weigh 0.53135 g of KHP and dissolve in \approx 200 mL of ultrapure water.
 - c. Add 6 N HCl until pH > 2 (check pH using pH strips by pipetting solution onto pH paper).
 - d. Once pH is > 2, add the remaining ultrapure water until it reaches 250 mL.
 - e. Store at 4 °C for no longer than 28 days.
2. Prepare working standard
 - a. 20 mg/L, 25 mg/L, 125 mg/L

Sample Preparation/Analysis

1. Filter 30 mL of sample through 0.45 μ m glass fiber filter.
 - a. Make sure filter vacuum is thoroughly cleaned and dried, void of organic contaminants.
 - b. If samples are turbid, homogenize the samples for 1 minute before filtering.
 - c. If filtered sample is still visibility turbid, dilute it with ultrapure water (note the dilution factor).
2. Transfer filtered samples to ashed glass TOC vials and add small magnetic Teflon stir bar.
3. Run samples immediately.

QA/QC

1. Includes blank, ultrapure water, pass if < 0.5 mg/L
2. Matrix spike, spike concentration varies each run, pass if within 25% recovery
3. Standard, concentration varies each run, pass if within 10% of expected value.
4. Triplicates

Alkalinity (HACH/EPA Method 8203)

1. Select the sample volume and Sulfuric Acid (H₂SO₄) Titration Cartridge corresponding to the expected alkalinity concentration as mg/L calcium carbonate (CaCO₃) from Table 3.
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.
3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.
4. Use a graduated cylinder or pipet to measure the sample volume from Table 3. Transfer the sample into a clean 250-mL Erlenmeyer flask. Dilute to about the 100-mL mark with deionized water, if necessary.
5. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix.
6. If the solution turns pink, titrate to a colorless end point. Place the delivery tube tip into the solution and swirl the flask while titrating with sulfuric acid. Record the number of digits required.
7. Calculate: Digits Required x Digit Multiplier = mg/L CaCO₃ Alkalinity.
8. Add the contents of one Bromocresol Green-Methyl Red Indicator Powder Pillow to the flask and swirl to mix.
9. Continue the titration with sulfuric acid to a light greenish blue-gray (pH 5.1), a light violet-gray (pH 4.8), or a light pink (pH 4.5) color, as required by the sample composition; see Table 4. Record the number of digits required.
10. Calculate: Total Digits Required x Digit Multiplier = mg/L as CaCO₃ Total Alkalinity

Table A3-3. Titration Cartridge corresponding to the expected alkalinity concentration as mg/L calcium carbonate (CaCO₃).

| Range (mg/L as CaCO ₃) | Sample Volume (mL) | Titration Cartridge (H ₂ SO ₄) | Catalog Number | Digit Multiplier |
|---------------------------------------|-----------------------|---|-------------------|---------------------|
| 10-40 | 100 | 0.1600 | 14388-01 | 0.1 |
| 40-160 | 25 | 0.1600 | 14388-01 | 0.4 |
| 100-400 | 100 | 1.600 | 14389-01 | 1.0 |
| 200-800 | 50 | 1.600 | 14389-01 | 2.0 |
| 500-2000 | 20 | 1.600 | 14389-01 | 5.0 |
| 1000-4000 | 10 | 1.600 | 14389-01 | 10.0 |

Table A3-4. Sample Composition and expected end point for alkalinity.

| Sample Composition | End Point |
|------------------------------------|--------------|
| Alkalinity about 30 mg/L | pH 4.9 |
| Alkalinity about 150 mg/L | pH 4.6 |
| Alkalinity about 500 mg/L | pH 4.3 |
| Silicates or Phosphates present | pH 4.5 |
| Industrial waste or complex system | pH 4.5 |

Methods resources

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Appendix 4: Data tables

Table A4-1: Wetlands microbial water quality

| | Total coliforms | | Fecal coliforms | | Enterococci | | Salmonella | |
|---------|-----------------|-------|-----------------|------|-------------|------|------------|------|
| | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | |
| Sample | in | out | in | out | in | out | in | out |
| 1 (wet) | 1986.3 | 117.8 | 547.5 | 51.2 | >2419.6 | 39.2 | 2.4 | 0.68 |
| 2 (wet) | >2419.6 | 17.3 | 547.4 | 28.3 | >2419.6 | 19.5 | 1.175 | 8.75 |

Table A4-2: Wetlands inorganic constituents

| | Cl ⁻ | | Br ⁻ | | SO ₄ ²⁻ | | I ⁻ | | NO ₂ ⁻ | | NO ₃ ⁻ | | PO ₄ ³⁻ | |
|---------|-----------------|-----|-----------------|-----|-------------------------------|------|----------------|-----|------------------------------|-------|------------------------------|-------|-------------------------------|------|
| | mg/L | | mg/L | | mg/L | | mg/L | | mg/L as N | | mg/L as N | | mg/L | |
| Sample | in | out | in | out | in | out | in | out | in | out | in | out | in | out |
| 1 (wet) | 20.3 | 148 | 0 | 0 | 34.6 | 72.3 | 0 | 0 | 0.013 (BDL) | 0.049 | 4.39 | 1.47 | 0.02 | 0.02 |
| 2 (wet) | | | | | | | | | 0.036 | 0.349 | 4.58 | 0.719 | 0.06 | 0.04 |

Table A4-3: Wetlands general water quality

| | TOC | | BOD ₅ | | TSS | | Conductivity | | pH | | Alkalinity | | Total Nitrogen | | Total Phosphorus | | COD | |
|---------|------|-----|------------------|-----|------|-----|--------------|-----|------|------|---------------------------|-----|----------------|------|------------------|------|------|------|
| | mg/L | | mg/L | | mg/L | | μS | | | | mg/L as CaCO ₃ | | mg/L | | mg/L | | mg/L | |
| Sample | in | out | in | out | in | out | in | out | in | out | in | out | in | out | in | out | in | out |
| 1 (wet) | 8.6 | 7.1 | 5.6 | 3.7 | 293 | 12 | 616 | 921 | 6.90 | 6.97 | 143 | 204 | 8.33 | 2.67 | 4.24 | 1.97 | 27.0 | 31.0 |
| 2 (wet) | 4.6 | 6.5 | 2.3 | 2.2 | 24 | 0.6 | 934 | 977 | 7.92 | 7.15 | 168 | 183 | 2.03 | 1.33 | 3.72 | 3.71 | 63.7 | 65.0 |

Table A4-4: AWPS microbial water quality

| | Total coliforms | | Fecal coliforms | | Enterococci | | Salmonella | |
|---------|-----------------|-----|-----------------|-----|-------------|-----|------------|--------|
| | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | |
| Sample | in | out | in | out | in | out | in | out |
| 1 (wet) | >2419.6 | 0 | >2419.6 | 0 | >2419.6 | 0 | 8.75 | <0.045 |
| 2 (wet) | >2419.6 | 0 | >2419.6 | 0 | >2419.6 | 0 | 27 | <0.045 |

Table A4-5: AWPS inorganic constituents

| | Cl ⁻ | | Br ⁻ | | SO ₄ ²⁻ | | I ⁻ | | NO ₂ ⁻ | | NO ₃ ⁻ | | PO ₄ ³⁻ | |
|---------|-----------------|------|-----------------|--------|-------------------------------|------|----------------|-----|------------------------------|------|------------------------------|-------|-------------------------------|------|
| | mg/L | | mg/L | | mg/L | | mg/L | | mg/L as N | | mg/L as N | | mg/L | |
| Sample | in | out | in | out | in | out | in | out | in | out | in | out | in | out |
| 1 (wet) | 227 | 4.54 | 0.115 | 0.0242 | 216 | 1.62 | 0 | 0 | 0.22 | 0.00 | 10.1 | 1.02 | 0.02 | 0.01 |
| 2 (wet) | | | | | | | | | 0.00 | 0.00 | 8.78 | 0.990 | 0.04 | 0.01 |

Table A4-6: AWPS general water quality

| | TOC | | BOD ₅ | | TSS | | Conductivity | | pH | | Alkalinity | | Total Nitrogen | | Total Phosphorus | | COD | |
|---------|------|-----|------------------|-----|------|-----|--------------|------|------|------|---------------------------|-----|----------------|------|------------------|------|------|-----|
| | mg/L | | mg/L | | mg/L | | μS | | | | mg/L as CaCO ₃ | | mg/L | | mg/L | | mg/L | |
| Sample | in | out | in | out | in | out | in | out | in | out | in | out | in | out | in | out | in | out |
| 1 (wet) | 7.6 | 0.1 | 7.0 | 0.3 | 4.5 | 0.8 | 1498 | 41.3 | 7.19 | 6.27 | 127 | 6.8 | 21.7 | 13.0 | 1.75 | 0.06 | 67 | 4.3 |
| 2 (wet) | 10.9 | 0.1 | 11.9 | BDL | 7.4 | 0.2 | 1590 | 29.2 | 7.16 | 5.67 | 150 | 4.3 | 11.3 | 1.7 | 2.26 | 0.10 | 121 | 15 |

Table A4-7: Groundwater recharge monitoring well microbial water quality

| Sample | Total coliforms | | Fecal coliforms | | Enterococci | | Salmonella | |
|---------|-----------------|--|-----------------|--|-------------|--|------------|--|
| | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | | MPN/100 mL | |
| 1 (wet) | 0 | | 0 | | 0 | | <0.045 | |
| 2 (wet) | 0 | | 0 | | 0 | | <0.045 | |

Table A4-8: Groundwater recharge monitoring well inorganic constituents

| Sample | Cl ⁻ | Br ⁻ | SO ₄ ²⁻ | I ⁻ | NO ₂ ⁻ | NO ₃ ⁻ | PO ₄ ³⁻ |
|---------|-----------------|-----------------|-------------------------------|----------------|------------------------------|------------------------------|-------------------------------|
| | mg/L | mg/L | mg/L | mg/L | mg/L as N | mg/L as N | mg/L |
| 1 (wet) | 5.76 | 0.00 | 0 | 0 | BDL | 1.50 | 0.01 |
| 2 (wet) | 5.54 | 0.07 | 1.91 | | 0.018 | 1.48 | 0.01 |

Table A4-9: Groundwater recharge monitoring well general water quality

| Sample | TOC | BOD ₅ | TSS | Conductivity | pH | Alkalinity | Total Nitrogen | Total Phosphorus | COD |
|---------|------|------------------|------|--------------|------|---------------------------|----------------|------------------|------|
| | mg/L | mg/L | mg/L | μS | | mg/L as CaCO ₃ | mg/L | mg/L | mg/L |
| 1 (wet) | 0.6 | BDL | 0.8 | 89.3 | 7.71 | 30.3 | 7.00 | 0.87 | 7.0 |
| 2 (wet) | 0.8 | 1.0 | 0.1 | 99.2 | 7.06 | 29.8 | 1.67 | 0.63 | 4.3 |

Table A4-10: Lake microbial water quality

| | Total coliforms | | | Fecal coliforms | | | Enterococci | | | Salmonella | | |
|---------|-----------------|------|-------|-----------------|-----|-----|-------------|-----|------|------------|-----|-------|
| | MPN/100 mL | | | MPN/100 mL | | | MPN/100 mL | | | MPN/100 mL | | |
| Sample | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW |
| 1 (dry) | 1 | 1 | 3 | 1 | 0 | 3.1 | 0 | 0 | 0 | 0.23 | 0.1 | 0.65 |
| 2 (dry) | 13.4 | 2 | 1 | 9.8 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.1 | 0.225 |
| 3 (wet) | 9.6 | 14.8 | 129.1 | 3.1 | 9.6 | 82 | 0 | 0 | 20.3 | 0.09 | 1.3 | 46 |

Table A4-11: Lake inorganic constituents

| | NO ₂ ⁻ | | | NO ₃ ⁻ | | | PO ₄ ³⁻ | | |
|---------|------------------------------|-----|-----|------------------------------|-------|-------|-------------------------------|------|------|
| | mg/L as N | | | mg/L as N | | | mg/L | | |
| Sample | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW |
| 1 (dry) | BDL | BDL | BDL | 4.15 | 0.226 | 0.241 | 0.01 | 0.20 | 0.01 |
| 2 (dry) | BDL | BDL | BDL | 4.64 | 0.273 | 0.265 | 0.02 | 0.02 | 0.02 |

Table A4-12: Lake general water quality

| | TOC | | | BOD ₅ | | | TSS | | | Conductivity | | |
|---------|------|-----|-----|------------------|-----|-----|------|-----|-----|--------------|------|------|
| | mg/L | | | mg/L | | | mg/L | | | μS | | |
| Sample | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW |
| 1 (dry) | 7.2 | 2.0 | 1.8 | 1.0 | 0.4 | 0.4 | 0.6 | 1.4 | 1.0 | 502 | 61.9 | 59.2 |
| 2 (dry) | 8.3 | 1.7 | 1.6 | 1.6 | | 1.0 | 1.6 | 2.6 | 1.2 | 483 | 65.1 | 65.4 |
| 3 (wet) | 7.1 | 1.8 | 2.2 | | | | | | | | | |

| | pH | | | Alkalinity | | | Total Nitrogen | | | Total Phosphorus | | | COD | | |
|---------|------|------|------|---------------------------|------|------|----------------|------|------|------------------|------|------|------|-----|----|
| | | | | mg/L as CaCO ₃ | | | mg/L | | | mg/L | | | mg/L | | |
| Sample | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW | WW | Mix | DW |
| 1 (dry) | 7.61 | 7.22 | 6.83 | 121 | 12.3 | 13.0 | 5.00 | 2.33 | 0.00 | 0.87 | 0.18 | 0.11 | 46 | 15 | 12 |
| 2 (dry) | 7.06 | 7.16 | 6.80 | 107 | 13.7 | 13.0 | 3.33 | 4.33 | 0.67 | 0.45 | 0.27 | 0.14 | 52 | 11 | 14 |

Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands

Basic Information

| | |
|---------------------------------|--|
| Title: | Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands |
| Project Number: | 2016NC209B |
| Start Date: | 9/1/2016 |
| End Date: | 8/31/2017 |
| Funding Source: | 104B |
| Congressional District: | NC-04 |
| Research Category: | Water Quality |
| Focus Categories: | Agriculture, Wetlands, None |
| Descriptors: | None |
| Principal Investigators: | Michael Burchell, Jalmar Kurki-Fox |

Publications

1. Messer, T.M., M.R. Burchell, and F. Birgand. 2017. Nitrate removal potential of restored wetlands loaded with agricultural drainage water: A mesocosm scale experimental approach. Ecological Engineering. 106:541-554.
2. Messer, T.M., M.R. Burchell, and F. Birgand. 2017. Comparison of four nitrate removal kinetic models in two distinct wetland restoration mesocosm systems. Water. 9(7) 517
doi:10.3390/w9070517
3. Messer, T.M., M.R. Burchell, J.K. Bohlke, and C. Tobias. 2017. Tracking the fate of nitrate through pulse-flow wetlands: A mesocosm scale ¹⁵N enrichment tracer study. Ecological Engineering. 106:597-608.

Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands

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Department of Biological and Agricultural Engineering

WRI Project # 16-13-W

May 16, 2018

1. SUMMARY

1.1 Activities and Findings

The project has encountered significant delays due the landowner's inaction on installing a new water control structure (WCS) at the restored wetland site and cleaning out other WCSs downstream from the site. However, in late June 2017 the new WCS was installed at the cell outlet. Heavy rains combined with poor communication delayed any further work for several weeks. In August of 2017, the water control structures and canals were cleaned out to prepare for pumping. The site was instrumented with flow and level monitoring sensors (Figure 1 and 2) and an adjustable weir was fabricated and installed at the outlet WCS (Figure 2) to control drawdowns. In addition, automatic water quality samplers were installed at the site (Figure 1 and 2).

Ready to pump to the cells for the first time in late August 2017, the landowners mistakenly diverted water away from the cell into other areas of the farm used for duck impoundments. Soon after, the precipitation pattern changed from wet to dry, and there was not enough drainage water to recharge the pumping canal. These conditions continued through the Fall of 2017. Then winter arrived. Record cold in the region made conducting studies such as this implausible.

To date, no pumping and drawdown trials have been completed. However, the equipment is in place to begin the tests. Trials are planned to begin in early April of 2018 and the landowner has placed different personnel in charge of the project to ensure better collaboration and communication. Activities completed to date include:

- Canals and WCSs cleaned out.
- Adjustable weir fabricated for outlet WCS.
- Hydrology and water quality monitoring equipment has been installed at the inlet and outlet of the wetland cell.
 - Inlet: HOBO water level logger, rain gauges, ISCO water quality sampler, and ISCO area velocity meter in culvert.
 - Outlet: Adjustable weir, HOBO water level logger, rain gauges, ISCO water quality sampler, and ISCO level probe.



Figure 1. Outlet monitoring equipment. a) Water quality sampler enclosure (green box), outlet WCS with weir and staff gage and intake. b) Sampler intake, ISCO level probe, staff gage, and HOBO level sensor.



Figure 2. Inlet monitoring equipment. a) Water quality sampler enclosure (green box), rain gauges, staff gauge and HOBO level sensor. b) Inlet WCS with sampler intake and ISCO area velocity meter installed.



Figure 3. Adjustable weir installed in outlet WCS. a) Weir in closed position and b) weir plate lowered to allow outflow (open position).

1.2 Deviations from Original Project Plans

The project site has been moved to a different restored wetland cell about two miles away from the original site. The new restored wetland cell is smaller than the previous site (50 ac. Vs. 300 ac.), which will allow for more accurate quantification of the results. The original cell's topography would have led to significant short-circuiting, and unforeseen hydraulic constraints downstream of the restored cell may have prevented the cell from draining adequately. The project has been expanded with additional funding to include monitoring bacteria in the wetland cell and surrounding waters. The project has been extended through December of 2018.

2. REFERENCES

N/A

3. APPENDIX 1

N/A

4. APPENDIX 2

N/A

nd use characteristics affect the prevalence of antibiotic resistant, virulent E. coli and host-specific markers in watersheds w

Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent E. coli and host-specific markers in watersheds with and without swine CAFOs

Basic Information

| | |
|---------------------------------|---|
| Title: | Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent E. coli and host-specific markers in watersheds with and without swine CAFOs |
| Project Number: | 2016NC210B |
| Start Date: | 9/1/2016 |
| End Date: | 8/31/2017 |
| Funding Source: | 104B |
| Congressional District: | NC-04 |
| Research Category: | Biological Sciences |
| Focus Categories: | Water Quality, None, None |
| Descriptors: | None |
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Title: Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent *E. coli* and host-specific markers in watersheds with and without swine CAFOs

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Introduction

The US Environmental Protection Agency (EPA) cites pathogens as the largest cause of surface water impairment, and EPA attributes the most probable source to agriculture from field runoff (1). In confined animal feeding operations (CAFOs) housing swine, large volumes of manure are typically stored in deep, open-air lagoons and subsequently sprayed onto sprayfields as fertilizer for crops. Without proper nutrient management and manure application procedures, nutrients and pathogens can leach into groundwater or be transported to surface water as runoff.

North Carolina (NC) is the second largest producer of swine in the United States (2) and CAFOs have been identified as a nonpoint source pollutant for nutrients in the surface water of our state (3, 4). Nutrient management and responsible application of manure is necessary to prevent surface water runoff or groundwater infiltration (2). Previous work from the USGS (5) compared small NC watersheds with CAFOs and those without CAFOs and identified “measurable effects of CAFO waste manures on stream water quality” by measuring nutrient content and also found that some land use characteristics such as a higher density of wetlands had a protective effect against nutrient contamination. However, this study did not measure the effect of CAFO presence in the watershed on water quality with respect to pathogens, source of pathogens, or antimicrobial resistance.

Although NC Department of Environmental Quality (DEQ) regulates nutrients from CAFOs they do not regulate microbial transport. Chronic microbial contamination has been identified in southeastern NC related directly to swine CAFOs (4). Testing surface water for fecal indicator bacteria, such as *E. coli*, is commonly used as an indicator for fecal pollution and human pathogen exposure (6, 7). Enterohemorrhagic *E. coli* (EHEC) are virulent strains of *E. coli* in humans with a low infectious dose that can cause severe gastrointestinal illness and has caused waterborne outbreaks (2). EHEC can originate in swine and one study found viable EHEC in surface water after a large swine manure spill (8). Additionally, a recently developed technique, microbial source tracking (MST), enables source identification of fecal contamination by targeting a gene “marker” specific to the host source (e.g. swine, chicken, or human). MST can help determine whether fecal contamination detected in water originated from swine or a different source (see, e.g., (9–11)). Additionally, droplet digital polymerase chain reaction (ddPCR) can be used to quantify the MST markers rather than testing only their presence or absence. ddPCR is a novel technology that splits a sample into many droplets and identifies the presence or absence of the target gene in each droplet (12). The output is a proportion of positive target gene reads. ddPCR has fewer technical barriers than standard curve-based real time PCR (qPCR) for quantification, as it is not as easily affected by inhibition, making it especially useful for environmental samples (12, 13). In NC, although swine-specific MST markers have been validated in swine manure (14), and although the transport of pathogens from swine CAFOs to surface water is documented, no studies have compared the presence of pathogenic *E. coli* and host-specific MST markers between watersheds with different land use relating to industrial swine production.

Finally, in addition to pathogen transport from livestock operations, antibiotic resistance can be passed to human pathogens (15) and so the transport of antibiotic resistant (ABR) bacteria from CAFOs is of concern (16). Current agricultural practices commonly administer antibiotics to

livestock to treat and prevent disease, and an estimated 75% of antibiotics are not absorbed but pass directly into the waste (16).

This study systematically compares watersheds with different land use characteristics, primarily with respect to swine CAFOs, and their effect on microbial water quality. The hypothesis was that antibiotic resistant *E. coli*, virulent *E. coli*, and swine-specific MST markers would be higher in watersheds with swine CAFOs compared to similarly sized agricultural watersheds without any CAFOs. The specific research objectives of this project were to (1) quantify microbial pollution as defined by *E. coli* concentration, (2) determine antibiotic resistance of *E. coli*, (3) determine virulence of *E. coli*, and (4) quantify microbial source tracking (MST) indicators to identify sources of *E. coli* between watersheds with and without swine CAFOs in NC.

Methods

Surface waters were collected and tested for selected microbial endpoints over the course of a year in watersheds with and without swine CAFOs. Sample selection was based on an earlier USGS study (5) that compared nutrient contamination at these same sites. In total, nine background sites and thirteen swine sites were sampled up to nine times between August 2016 and August 2017 for a total of 196 sampling events. Background sites were defined as having an upstream watershed land area that is primarily agricultural land and does not contain any type of CAFO or wastewater treatment plant. Swine sites were defined as having an upstream watershed land area that is primarily agricultural land and contains a swine CAFO barn and/or lagoon and/or sprayfield and does not have any other kind of CAFO or wastewater treatment plant. Approximately one liter of water was collected at each sampling site and sampling time and transferred on ice to the laboratory at the University of North Carolina at Chapel Hill. Samples were processed at the laboratory within 24 hours of sample collection.

***E. coli* culture and quantification**

Standard membrane filtration methods were used to quantify concentrations of *E. coli* from each water sample collected (17). Briefly, 50mL, 25mL, 5mL, and 1mL volumes of water were filtered through four membranes and the membranes were aseptically placed on plates containing selective mTEC media. The plates are inverted and incubated at 37°C for 2 hr followed by 44 °C for 22 hr (+/- 2 hr) then colonies with morphological characteristics of *E. coli* were summed and used to calculate concentrations of colony forming units (CFUs) per 100mL. CFU was determined by averaging the CFU among countable dilutions. Up to five *E. coli* colonies per sample were then isolated, purified, confirmed through biochemical testing including indole production, and archived for further analysis.

Antimicrobial resistance testing

Antimicrobial resistance testing was conducted on all archived *E. coli* isolates using standard Kirby-Bauer disc diffusion methods and following standard CLSI guidelines (18). Isolates were tested for resistance to eleven antibiotics comprising nine antibiotic classes as recommended by NARMS (19) and CLSI (18) guidelines. Tested antibiotics included antibiotics used primarily in industrial agriculture (20) and antibiotics used primarily in human medicine (21) with risk assessment priority levels assigned based on WHO criteria (22) (Table 1). Multi-drug resistance was defined as resistance to three or more antibiotic classes. Isolates were also screened for

carbapenem resistance, AmpC β -lactamase production, and extended spectrum β -lactamase (ESBL) production, which are resistance traits of high public health concern. For this study, a positive screen for carbapenem resistance was resistance to imipenem, for AmpC β -lactamase production was resistance to cefoxitin, and for ESBL production was intermediate or complete resistance to ceftriaxone. Isolates with a positive screen for AmpC β -lactamase production were confirmed through the disc approximation test (23) while isolates with a positive screen for ESBL production were confirmed using CLSI protocol (18).

Table 1: Antibiotics included in antimicrobial resistance testing of *E. coli*

| Antibiotic | Antibiotic Class | Veterinary Use | Human Use | WHO Priority |
|-------------------------------|-------------------|----------------|-----------|---------------------------|
| Amoxicillin-Clavulanate Acid | Penicillin | Yes | Yes | High Priority Critical |
| Ampicillin | Penicillin | Yes | Yes | High Priority Critical |
| Cefoxitin | Cephalosporin | No | Yes | Highly Important |
| Ceftriaxone | Cephalosporin III | No | Yes | Highest Priority Critical |
| Chloramphenicol | Amphenicol | Yes | Yes | Highly Important |
| Ciprofloxacin | Fluoroquinolones | No | Yes | Highest Priority Critical |
| Gentamicin | Aminoglycosides | Yes | Yes | High Priority Critical |
| Imipenem | Carbapenem | No | Yes | High Priority Critical |
| Levofloxacin | Fluoroquinolones | No | Yes | Highest Priority Critical |
| Tetracycline | Tetracyclines | Yes | Yes | Highly Important |
| Trimethoprim-Sulfamethoxazole | Sulfas | No | Yes | Highly Important |

Virulence testing

E. coli isolates were prioritized for virulence characterization if they were multi-drug resistant or had a positive screen for carbapenem, AmpC, or ESBL production. Virulence testing was conducted at the NCSU Clinical Microbiology Laboratory under the direction of Dr. Megan Jacob. Prioritized *E. coli* isolates were characterized using a multiplex polymerase chain reaction (PCR) assay for six virulence genes (*stx1*, *stx2*, *hlyA*, *rfbE*, *eae*, *fliC*) associated with the human pathogen *E. coli* O157:H7 (24) as well as two genes, *CMY2* and *TEM*, associated with ESBL production (25).

Microbial source tracking

During sample processing, 100mL of each sample were filtered through 0.45um polycarbonate filters and saved in MoBio PowerSoil DNA extraction tubes (Qiagen Inc, Germantown, MD) at -80°C until extraction. DNA extraction using the PowerSoil kit was then conducted following manufacturer's protocol with one addendum that tubes were bead beaten for two minutes prior to extraction. Extracted DNA was then stored frozen at -80°C until ddPCR analysis which was conducted as a duplex assay targeting *pig2bac* (14, 26), associated with swine fecal contamination, and *Bacteroides* HF183, associated with human fecal contamination (11, 27). Prior to running samples, the duplex assays were optimized for reaction temperature and assessed for assay competition. All samples (n=196) were then run in duplicate for *pig2bac* and HF183 using the optimized parameters.

To quantify the MST targets, the range of values for a negative droplet was determined by finding the mean amplitude of droplets in negative samples (e.g. negative extraction controls, negative template controls, and field blanks) plus or minus two standard deviations. The range of values for a positive droplet was determined from sample standards by subtracting out mean negative concentration and finding the mean amplitude of positive droplets plus or minus two standard deviations. The 95% confidence interval lower bound of this range was considered the threshold value to determine positive droplets in samples. In samples, a droplet was considered positive if above the threshold value for the gene target.

Results

***E. coli* culture and quantification**

Of 196 total sampling events, 187 events were included in the quantitative, CFU analysis of *E. coli*. Four samples were not included in the CFU analysis because the site was dry at the time of sample collection, and five samples were excluded due to a laboratory error in culturing *E. coli*. Table 2 identifies the number of times a site was sampled, the number of times a CFU was determined, and the total number of confirmed *E. coli* isolates that were archived for each site. Sites were sampled an average of 8.5 times each between August 2016 and August 2017 with an average of 40 *E. coli* isolates archived for each background site and 43 *E. coli* isolates archived for each swine site.

Table 2. Sampling summary for 22 sites between August 2016 and August 2017 including the number of sites sampled, number of sampling events per site, number of times CFU was determined, and number of *E. coli* isolates archived.

| Site | n sample dates | n CFU determined | n <i>E. coli</i> isolates |
|-------------|---------------------------|-----------------------------|--------------------------------------|
| BK01U | 9 | 9 | 47 |
| BK03 | 9 | 9 | 45 |
| BK05U | 9 | 9 | 40 |
| BK10U | 9 | 8 | 24 |
| BK12 | 9 | 9 | 38 |
| BK14 | 9 | 9 | 43 |
| BK15 | 9 | 9 | 43 |
| BK16 | 9 | 9 | 41 |
| BK17U | 9 | 6 | 35 |
| SW01 | 9 | 9 | 46 |
| SW04 | 9 | 9 | 46 |
| SW05 | 9 | 9 | 43 |
| SW05A | 9 | 8 | 43 |
| SW05C | 9 | 9 | 41 |
| SW07 | 9 | 9 | 44 |
| SW09 | 8 | 8 | 39 |

| Site | n sample dates | n CFU determined | n <i>E. coli</i> isolates |
|-------|----------------|------------------|---------------------------|
| SW10 | 9 | 9 | 45 |
| SW11 | 9 | 9 | 45 |
| SW13 | 8 | 8 | 40 |
| SW16 | 9 | 8 | 43 |
| SW17 | 9 | 8 | 39 |
| SW17U | 9 | 7 | 42 |

In total, 912 *E. coli* isolated from swine and background sites were confirmed as indole producers and have been archived. Figure 1 displays a boxplot of *E. coli* concentrations identified at all sites. The y-axis in Figure 1 is cut at 9000 CFU/100mL although a few concentrations are above this value. Additionally, among sampling events, 42% (n=77) of background sites and 70% (n=110) swine sites were above the EPA recommendation of 126 CFU/100mL for recreational waters.

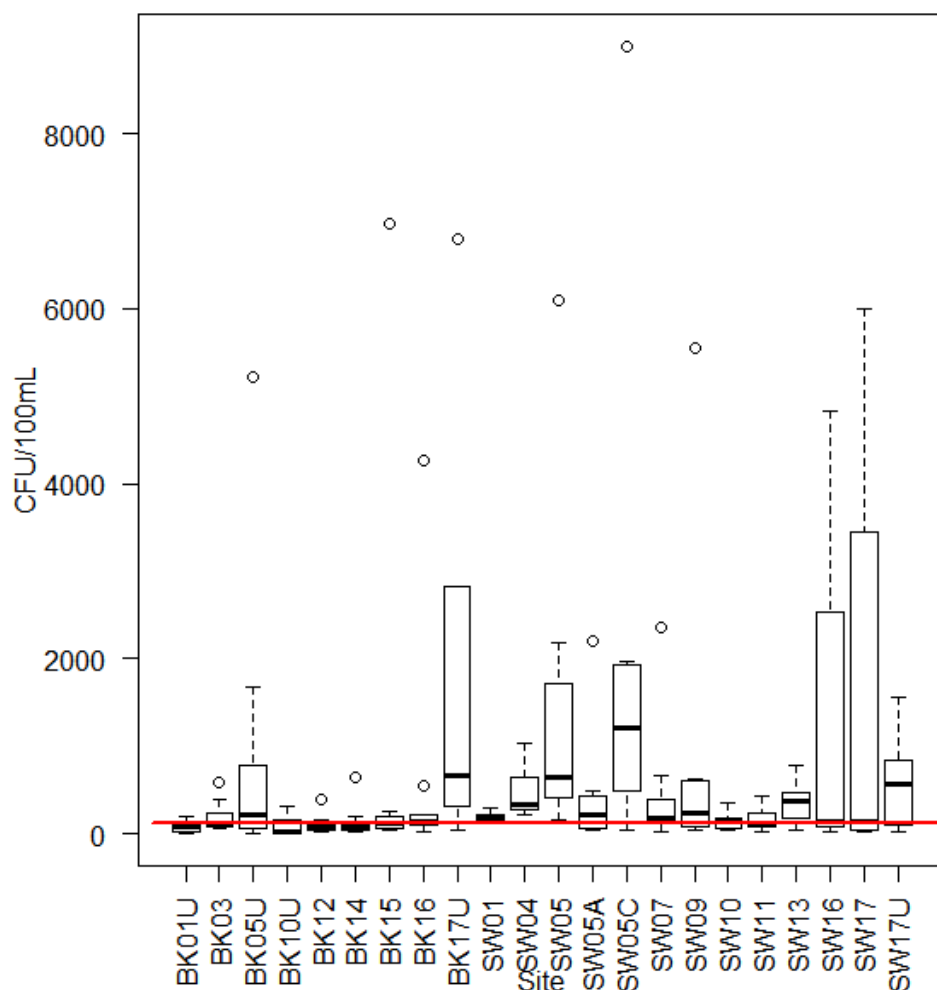


Figure 1: *E. coli* concentrations at all sites throughout sampling period. The red line represents the 2012 EPA recommendation for recreational waters of 126 CFU/100mL.

Table 3 identifies mean, minimum, and maximum *E. coli* concentrations for each site. Mean concentrations of *E. coli* in background sites was 501 CFU/100mL (95% confidence interval (CI)= 203-800) compared to mean concentrations of 1188 CFU/100mL (CI= 522-1854) in swine sites.

Table 3: Concentrations of *E. coli* observed for each site including background (BK) and swine (SW) sites.

| Site | Average (CFU/100mL) | 95% Standard Error (CFU/100mL) | Minimum (CFU/100mL) | Maximum (CFU/100mL) |
|-------|------------------------|---|------------------------|------------------------|
| BK01U | 89 | 48 | 2 | 200 |
| BK03 | 202 | 116 | 56 | 580 |
| BK05U | 937 | 1106 | 10 | 5220 |
| BK10U | 83 | 70 | 0 | 306 |
| BK12 | 99 | 78 | 12 | 392 |
| BK14 | 145 | 127 | 18 | 640 |
| BK15 | 880 | 1493 | 40 | 6970 |
| BK16 | 625 | 896 | 26 | 4260 |
| BK17U | 1885 | 2089 | 42 | 6800 |
| SW01 | 193 | 34 | 124 | 292 |
| SW04 | 3719 | 6441 | 220 | 30000 |
| SW05 | 1485 | 1215 | 154 | 6100 |
| SW05A | 462 | 504 | 44 | 2210 |
| SW05C | 1874 | 1807 | 43 | 9000 |
| SW07 | 483 | 475 | 20 | 2350 |
| SW09 | 922 | 1303 | 40 | 5540 |
| SW10 | 155 | 81 | 35 | 358 |
| SW11 | 160 | 88 | 20 | 432 |
| SW13 | 357 | 159 | 47 | 780 |
| SW16 | 2714 | 3945 | 22 | 16200 |
| SW17 | 2308 | 2865 | 12 | 11200 |
| SW17U | 571 | 416 | 24 | 1550 |

Antimicrobial resistance testing

Of 912 *E. coli* isolates archived, 356 background and 556 swine *E. coli* isolates were tested for resistance to eleven antibiotics including screening for carbapenem resistance, AmpC β -lactamase production, and ESBL production. Table 4 displays the results of antimicrobial resistance testing. Antimicrobial resistance to at least one antibiotic was observed in isolates collected from swine sites (19%, n=556) more often than background sites (6%, n=356) (Table 4,

Figure 2). For every antibiotic with observed resistance, resistance was more often observed in isolates from swine sites compared to those from background sites. Tetracycline resistance was the most commonly observed with 17% of swine isolates compared to 5% of background isolates followed by ampicillin resistance in 5% swine isolates compared to 0.8% background isolates.

Table 4: Number and percent of *E. coli* isolates with observed resistance to antibiotics from water samples collected from background and swine sites. Observed resistance does not include observed intermediate resistance. Amoxicillin-clauvanate acid (AmC), ampicillin (AM), ceftriaxone (CRO), chloramphenicol (C), ciprofloxacin (CIP), ceftiofur (FOX), gentamycin (GM), imipenem (IPM), levofloxacin (LVX), sulfamethoxazole-trimethoprim (SXT), and tetracycline (TE).

| Sample Type | AmC | AM | CRO | C | CIP | FOX | GM | IPM | LVX | SXT | TE |
|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----|-------------|-------------|--------------|
| Background | 1 (0.3%) | 3 (0.8%) | 0 | 0 | 0 | 1 (0.3%) | 0 | 0 | 0 | 0 | 19 (5.4%) |
| Swine | 4 (7%) | 28 (5%) | 7 (1.3%) | 5 (0.9%) | 2 (0.4%) | 4 (0.7%) | 2 (0.4%) | 0 | 2 (0.4%) | 7 (1.3%) | 96 (17%) |

Figure 2 identifies the percent of isolates from swine and background sites and the number of antibiotic classes with observed resistance. Swine sites were more likely to be resistant to a higher number of antibiotic classes. Multi-drug resistance, defined as resistance to three or more classes of antibiotics, has been observed among 2.5% (n=556) of *E. coli* isolates from sites downstream from swine CAFOs, and from 0.28% (n=356) of *E. coli* from background sites (Figure 3). Multi-drug resistance was observed in one isolate from a background site compared to 14 isolates from four swine sites across twelve sampling events.

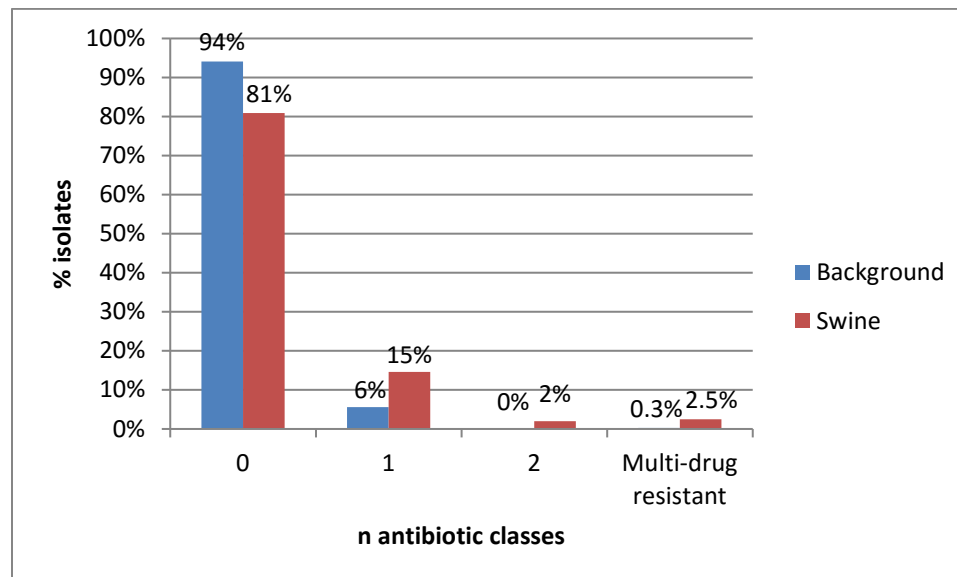


Figure 2: The percent of isolates from each observational group that are resistant to 0, 1, 2, or more (i.e. multi-drug resistant) classes of antibiotics.

Screens for carbapenem resistance, AmpC β -lactamase production, and ESBL production were conducted for all archived isolates (n=912). Results are summarized in Table 5. No isolates had a positive screen for carbapenem production since imipenem resistance was not observed. One isolate was confirmed as AmpC-producing and four isolates were confirmed as ESBL-producing. Confirmed AmpC and ESBL-producing isolates (n=5) were from swine sites.

Table 5: Results of multi-drug resistance, screening, and confirmation tests for carbapenem, AmpC, and ESBL production among *E. coli* isolates.

| Sample Type | Multi-Drug Resistant isolates (n) | Positive Screen (n) | | | Positive Confirmation (n) | |
|-------------|-----------------------------------|---------------------|------|------|---------------------------|------|
| | | Carbapenem | AmpC | ESBL | AmpC | ESBL |
| Background | 1 | 0 | 0 | 1 | 0 | 0 |
| Swine | 14 | 0 | 1 | 8 | 1 | 4 |

Virulence testing

Virulence testing was conducted on prioritized *E. coli* isolates (n=17) comprising all isolates with a positive screen for AmpC production (n=1) or ESBL production (n=9), and all isolates that were multi-drug resistant (n=15). Two isolates were tested that were not multi-drug resistant but had a positive screen for AmpC or ESBL production. An additional swine isolate remains to be tested for a total of 18 isolates that will be characterized. Of isolates prioritized for virulence testing, 16 were from swine sites and one was from a background site (Figure 3).

Isolates tested did not carry both ESBL genes *TEM* and *CMY2*, but rather 13 of the 17 isolates (76%) were positive for one of the two ESBL targets. Additionally, isolates with a positive confirmation of ESBL by culture did not have either ESBL gene target. One isolate from a site downstream of swine CAFOs was also positive for *stx2*, encoding shiga toxin production, and *hlyA*, encoding hemolysin toxin production (Table 6). These results show that virulence factors can be detected in waterborne *E. coli* isolates near swine CAFOs.

Table 6: Characterization of multi-drug resistant *E. coli* isolates from swine and background sites for extended-spectrum beta-lactamase (ESBL) production and genes associated with the human pathogen O157:H7.

| Sample Type | n tested | ESBL | | O157:H7 | | | | | |
|-------------|----------|------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|
| | | <i>TEM</i> | <i>CMY2</i> | <i>stx1</i> | <i>stx2</i> | <i>hlyA</i> | <i>rfbE</i> | <i>eae</i> | <i>flyC</i> |
| Background | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Swine | 16 | 8 | 4 | 0 | 1 | 1 | 0 | 0 | 0 |

| Site | 2016 | | | | | 2017 | | | | | | | |
|-------|------|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|
| | Aug | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug |
| SW01 | | & | | | | | | | | | | | |
| SW04 | | | * | | | | *# | | | * | | | |
| SW05 | * | | | * | * | | | | * | | | * | |
| SW05A | | | | | | | | | | | | | |
| SW05C | | | | | | | | | * | | * | * | |
| SW07 | | | | | | | | | | | | | |
| SW09 | | | | | * | | | | | | | | |
| SW10 | | | | | | | | | | | | | |
| SW11 | | | | | | | | | | | | | |
| SW13 | | | | | | | | | | | | | |
| SW16 | | | | | | | | | | | | | |
| SW17 | | | | | | | | | | | | | |
| SW17U | | | | | | | | | | | | | |
| BK01U | | | | | | | | | | | | | |
| BK03 | | | | | | | | | | | | | |
| BK05U | | | | | | | | | | | | | |
| BK10U | | | | | | | | | | | | | |
| BK12 | | | | | | | | | | | | | |
| BK14 | | | | | | | | | | | | | |
| BK15 | | | | | | | | * | | | | | |
| BK16 | | | | | | | | | | | | | |
| BK17U | | | | | | | | | | | | | |

| | |
|---|-----------------------|
| | No Sampling Event |
| | No Positive Result |
| | Multi-Drug Resistance |
| & | AmpC |
| * | ESBL |
| # | Other Virulence |

Figure 3: Sites and associated sampling events with at least one isolate positive for multi-drug resistance (grey) and/or virulence characteristics (AmpC - &, ESBL - *, and other - #). ESBL positivity is considered positive by culture or one of two genetic targets, and other virulence (#) indicates positivity for at least one of six virulence factors that characterize O157:H7.

Microbial source tracking

All samples (n=194) were run in duplicate for pig2bac and HF183 using the duplex ddPCR assay and data analysis is ongoing. Resulting gene target concentrations are subject to quality control steps such as ensuring that droplet generation is over 10,000 droplets and ensuring threshold values are based on positive and negative control ranges.

Discussion

In our landscape-scale watershed study of the effects of land use on the presence of virulent, antibiotic-resistant *E. coli* and microbial source markers, we found higher mean concentrations of *E. coli* at swine sites compared to background sites (1188 CFU/100mL (CI= 522-1845) vs. 503 CFU/100mL(CI= 203-800)), higher antibiotic resistance to at least one antibiotic at swine compared to background sites (19% vs. 6%), higher multi-drug resistance at swine sites compared to background sites (2.5% vs. 0.28%), and higher number of virulence factors at swine sites compared to background sites. Additionally, among sampling events, 42% (n=77) of background sites and 70% (n=110) swine sites were above the EPA recommendation of 126 CFU/100mL for recreational waters.

Our future work is to finish assessment of ddPCR microbial source tracking gene targets HF183 and pig2bac among samples and to use exploratory research techniques to assess potential relationships of spatial covariates with microbial outcomes. With variables of interest collected, we are now able to begin modeling efforts to identify the impacts of environmental variables including precipitation, distance to nearest sprayfield, percent wetland in watershed, steady state live weight of hogs permitted upstream of sampling point, and human population from census block data. Previous work has identified that geospatial data can be used in combination with water quality data to identify the source of nonpoint source pollution (5, 6). Land use characteristics, such as wetlands and vegetative river buffers, may help mitigate the effects of swine CAFOs on receiving surface waters. Wetlands have been shown to reduce pathogen and antimicrobial input into receiving surface waters from CAFOs (28, 29). Our future work will analyze continuous variables such as percent wetland together with discretely collected variables such as *E. coli* concentration in water samples and CAFO-specific manure application data from state permits.

Training:

This fellowship provided research funding for PhD student Elizabeth Christenson to collect the data presented which will form the basis of her dissertation. Implementation of this project also included training of five undergraduate students and two master's level students who assisted with field and laboratory work including sample collection, media preparation, membrane filtration, *E. coli* culture and isolation, and antibiotic resistance testing. One master's student leveraged water samples collected from this field project to assess antibiotic resistant *Staphylococcus aureus* for a subset of the swine and background samples collected, analysis that will form the basis of her MS thesis. This fellowship also provided funding for one PhD student and one master's level student to attend the annual WRI conference in Raleigh, NC in March 2017.

Students supported include the following:

Elizabeth Christenson* (PhD, in progress)

Ryan Leighton (BSPH 2017; MS, in progress)

Lindsay Wickersham* (BSPH 2017; MS, in progress)

Rachel Lempp (BSPH with honors, in progress)

Pooja Naik (BSPH, in progress)

Maddy Grace Ponder (BSPH, in progress)

Maggie Lucas (BS, in progress)

Matthew Herman (BS, 2017)

*using data collected from this project as basis for thesis or dissertation

Presentations:

Christenson, E., Stewart, J. Prevalence of antibiotic-resistant *E. coli* in North Carolina watersheds with and without swine CAFOs. UNC Water Microbiology Conference. Chapel Hill, NC. May 15-17, 2017. [poster]

Stewart, JR. Tracking pathogens in coastal waters. North Carolina Coastal Conference. Raleigh, NC. April 5, 2017.

Christenson, E., Stewart, J. Prevalence of antibiotic-resistant *E. coli* in North Carolina watersheds with and without swine CAFOs. Water Resources Research Institute annual conference. Raleigh, NC. March 16, 2017.

Stewart, JR. Water research at UNC: Assisting communities across NC. NC Clean Tech Summit. March 3. Chapel Hill, NC. March 3, 2017.

Christenson, E. All that glimmers is not gold: Understanding how land use characteristics affect the prevalence of antibiotic resistant *E. coli* in watersheds with and without swine CAFOs. Environmental Sciences and Engineering department seminar. Chapel Hill, NC. March 1, 2017.

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Tracing Groundwater Contamination Near And Away From Coal Ash Ponds in North Carolina

Basic Information

| | |
|---------------------------------|---|
| Title: | Tracing Groundwater Contamination Near And Away From Coal Ash Ponds in North Carolina |
| Project Number: | 2017NC211B |
| Start Date: | 3/1/2017 |
| End Date: | 2/28/2018 |
| Funding Source: | 104B |
| Congressional District: | NC-4 |
| Research Category: | Water Quality |
| Focus Categories: | Water Quality, Hydrogeochemistry, Toxic Substances |
| Descriptors: | None |
| Principal Investigators: | Avner Vengosh |

Publication

1. Vengosh, A., Coyte, R., Karr, J., Harkness, J.S., Kondash, A.J.*, Laura S. Ruhl, L.A., Rose B. Merola, R.B., Dywer, G.S. (2016) The Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina. Environmental Science & Technology Letters, 3 (12), 409–414.

The myth and reality of hexavalent chromium in drinking water wells in North Carolina and the connection to coal ash contamination

Avner Vengosh

Nicholas School of Environmental Sciences
Duke University
(vengosh@duke.edu)



The myth and reality of hexavalent chromium in drinking water wells in North Carolina and the connection to coal ash contamination



Kristen McKinley,
MEM student



Rachel Coyte,
PhD student



Jennifer Harkness,
PhD student (graduated 2017)

Funding: North Carolina Water Resources Research Institute

Hexavalent chromium is known to be a highly toxic and carcinogen...

| State / Agency | Threshold |
|---------------------------------------|---|
| EPA – federal Safe Drinking Water Act | Hexavalent chromium: N/A Total chromium: 100 ppb |
| NC 02L groundwater standard | Hexavalent chromium: N/A Total chromium: 10 ppb |
| NC DHHS health screening level | 0.07 ppb |
| California drinking water standard | 10 ppb |
| California public health goal | 0.02 ppb |

Dissolved chromium in water:

Trivalent chromium – Cr(III)
Hexavalent Chromium – Cr(VI)

North Carolina Department of Health and Human Services

April 2015 - letters to hundreds homeowners living near coal ash ponds

Do not drinking your well water !!!!!!!!

High hexavalent chromium (and vanadium)

Public concern: hexavalent chromium in drinking water wells is the result of coal ash pond contamination



The objectives of this study are:

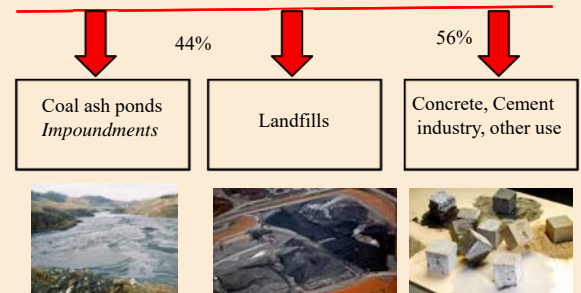
- Evaluation of the magnitude and speciation of chromium mobilization from coal ash;
- Determine whether groundwater contaminated with hexavalent chromium derived from nearby coal ash ponds leaking



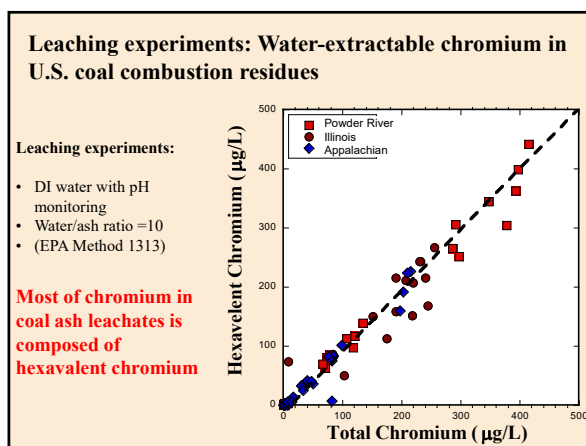
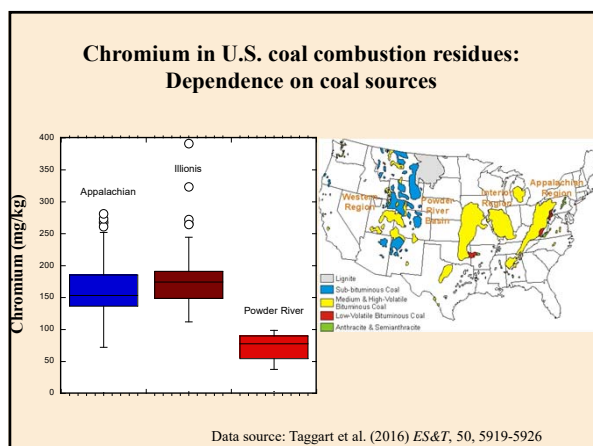
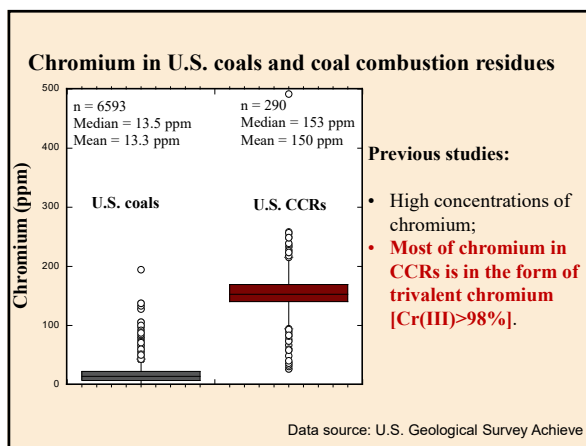
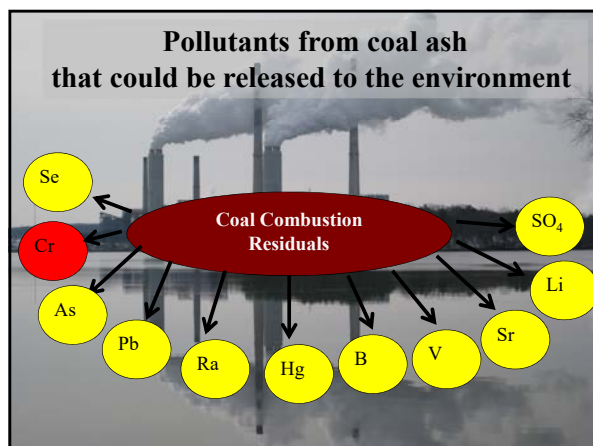
Five hundreds power plants nationwide generate approximately 130 million tons of coal ash each year



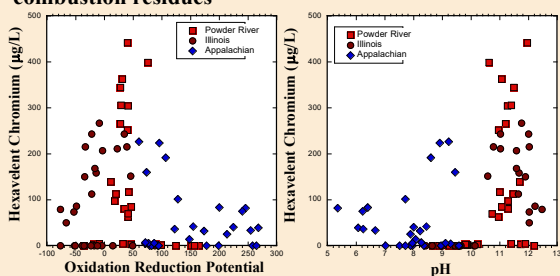
Disposal of Coal Combustion Residuals



2016 – total of 107.4 million short tons (American Coal Ash Association)

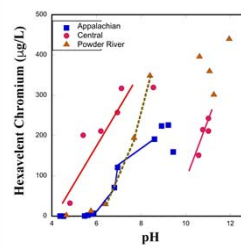


Results: Water-extractable chromium in U.S. coal combustion residues



The buffering capacity of CCRs: Cr(VI) in water leachates is restricted to alkaline and redox sweet spots...

Results: Water-extractable chromium in U.S. coal combustion residues



pH Control experiments: increase of Cr(VI) with pH

Evidence for coal ash effluents contamination of shallow groundwater

Harkness et al. (2016), *ES&T*, 50, 6583–6592.

The impact of coal ash ponds on water resources



ENVIRONMENTAL Science & Technology LETTERS

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Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina

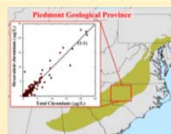
Avner Vengosh,^{1,2} Rachel Coyte,¹ Jonathan Karr,¹ Jennifer S. Harkness,¹ Andrew J. Kondash,¹ Laura S. Ruhl,¹ Rose B. Merola,¹ and Gary S. Dwyer¹

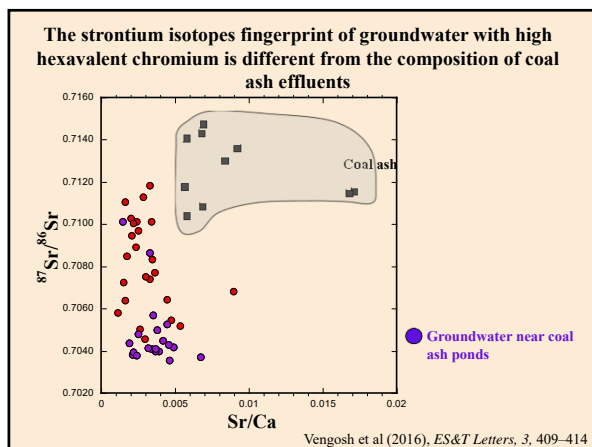
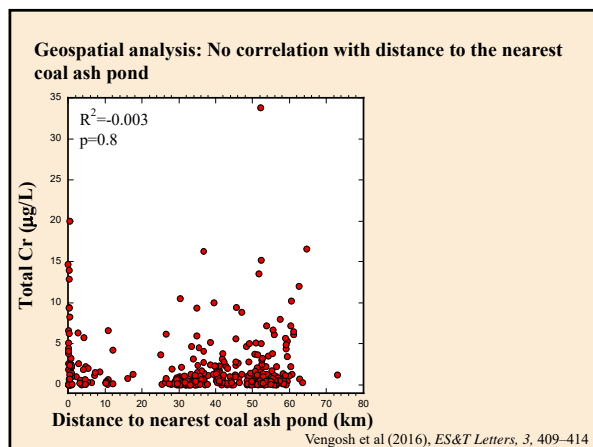
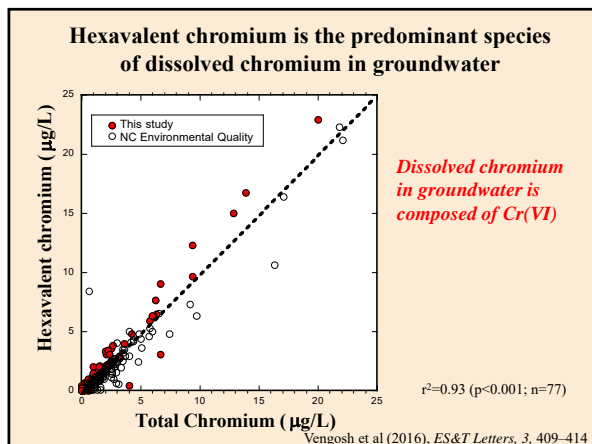
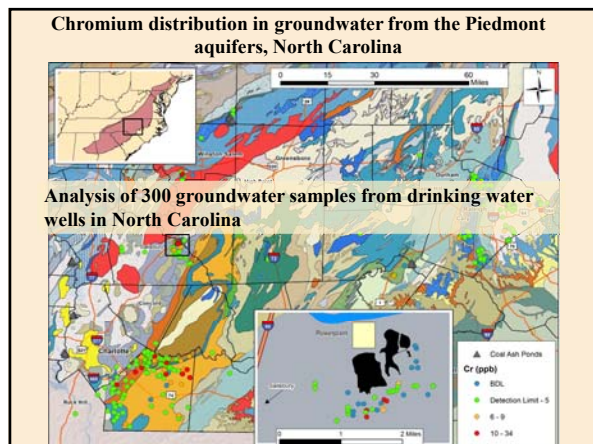
¹Division of Earth and Ocean Sciences, Nicholas School of the Environment, Duke University, Durham, North Carolina 27708, United States

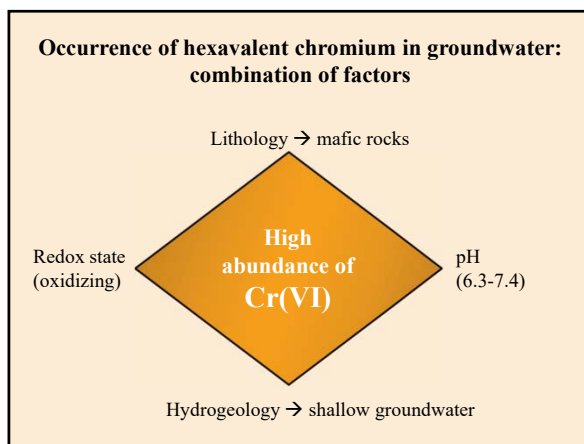
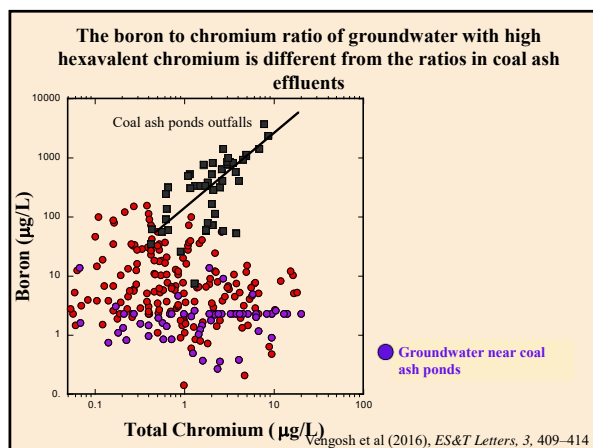
²Department of Earth Sciences, University of Arkansas at Little Rock, Little Rock, Arkansas 72204, United States

Supporting Information

ABSTRACT: Hexavalent chromium [Cr(VI)] is a known pulmonary carcinogen. Recent detection of Cr(VI) in drinking water wells in North Carolina has raised public concern about contamination of drinking water wells by nearby coal ash ponds. Here we report, for the first time, the prevalence of Cr and Cr(VI) in drinking water wells from the Piedmont region of central North Carolina, combined with a geochemical analysis to determine the source of the elevated Cr(VI) levels. We show that Cr(VI) is the predominant species of dissolved Cr in groundwater and elevated levels of Cr and Cr(VI) are found in wells located both near and far (>30 km) from coal ash ponds. The geochemical characteristics, including the overall chemistry, boron to chromium ratios, and strontium isotope (⁸⁷Sr/⁸⁶Sr) variations in groundwater with elevated Cr(VI) levels, are different from those of coal ash leachates. Alternatively, the groundwater chemistry and Sr isotope variations are consistent with water-rock interactions as the major source for Cr(VI) in groundwater. Our results indicate that Cr(VI) is most likely naturally occurring and ubiquitous in groundwater from the Piedmont region in the eastern United States, which could pose health risks to residents in the region who consume well water as a major drinking water source.



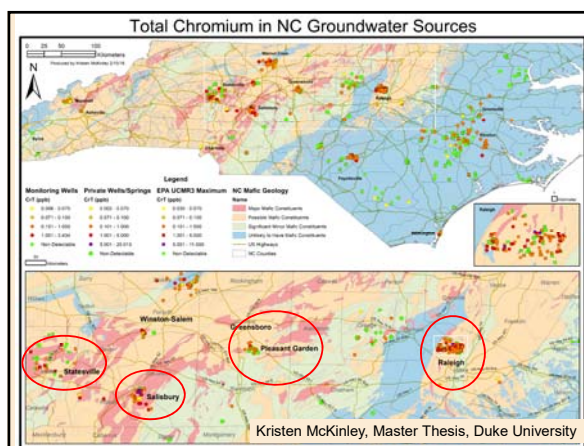


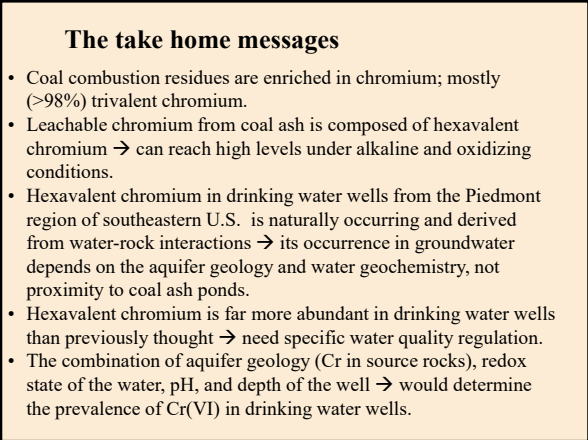


New dataset on hexavalent chromium in North Carolina groundwater

- Additional private wells sampling (total 479 wells; 180 data points of hexavalent chromium)
- Sampling of monitoring wells (with collaboration of Amy *Keyworth*, Division of Water Resources, Planning Section, NC Department of Environmental Quality) (total analyzed 73 wells);
- Domestic drinking water wells (from EPA archive data; n= 332 wells)

Kristen McKinley, Master Thesis, Duke University





How, where, when, and why: Defining Eutrophication Related Trends in Water Quality for the Middle and Lower Cape Fear River Basin

Basic Information

| | |
|---------------------------------|---|
| Title: | How, where, when, and why: Defining Eutrophication Related Trends in Water Quality for the Middle and Lower Cape Fear River Basin |
| Project Number: | 2017NC212B |
| Start Date: | 3/1/2017 |
| End Date: | 2/28/2018 |
| Funding Source: | 104B |
| Congressional District: | NC-3 |
| Research Category: | Water Quality |
| Focus Categories: | None, None, None |
| Descriptors: | None |
| Principal Investigators: | Nathan S Hall, Hans W Paerl |

Publications

There are no publications.

Please answer questions as they apply to activities between
March 1, 2017 and February 28, 2018
Please return to Nicole_Wilkinson@ncsu.edu by Monday, May 14, 2018.

Specific Reporting Questions:

1. A. Please specify the number of each of the following: undergraduates, masters students, PhDs and/or postdocs who worked on this project.

None.

B. Please also indicate in parentheses whether their field of study was engineering, physical sciences, biological sciences, natural resources/agricultural sciences, social sciences, or other (define other).

C. Please include the students' names.

D. Finally, please also indicate the number of faculty working on this project.

Nathan Hall and Hans Paerl

2. Did you or your students receive any awards or recognition for the research performed under this grant (example: a student received an honorable mention for a presentation delivered at a conference based on research results from this project)? If so, please specify the nature of the award (date, name of award, person receiving award, and organization/conference issuing the award).

None

3. Please list any presentations, workshops, technology demonstrations, outreach events etc delivered based on research results from this project (include date, title, location of presentation and/or conference, and a brief description of session). If a workshop was held, please include number of attendees.

PI Hall conveyed project findings to stakeholders through the Middle Cape Fear River Basin Association spring meeting (25 May 2017, Fayetteville, NC). The talk entitled "Trend analyses of water quality conditions along the middle Cape Fear River" presented an update of progress on determining water quality trends in the Middle Cape Fear, and generated discussion that led to a great deal of interest in determining trends of point source nutrient loads.

PI Hall gave an oral presentation entitled "Unraveling dual influences of increasing nutrients and changing flow regimes on bloom potentials along the middle Cape Fear River" at NC WRRI's annual conference (16 May 2017, Raleigh, NC).

PI Hall co-chaired a special session devoted to the influence of physical processes on cyanobacteria blooms at the Society for Freshwater Sciences annual conference (6 Jun. 2017,

Raleigh, NC), and gave an oral presentation on the physical drivers of blooms on the Cape Fear River entitled "Unraveling the multifaceted effects of changing flow regimes on cyanobacterial bloom potentials on the Cape Fear River, NC."

PI Hall gave an oral presentation at the 9th Annual US Symposium on Harmful Algae (Nov. 2017, Baltimore, MD) entitled "Unraveling the multifaceted effects of changing flow regimes and hydrologic modifications on cyanobacterial bloom potentials along the Cape Fear River, NC."

4. If student attendance at a conference was supported with funds from this project, please list the title of the conference and number of students who attended (note, this can be attendance only, and does not have to include giving a presentation, which would be covered under question 4).

None.

5. Were any teaching assistantships given as a result of the award?

None.

6. Have you received any additional grants or funding as a supplement to this award, or for which this research served as the foundation? If so, please provide the title of the new grant/project/award, source of funding, amount of funding received, who the project was awarded to (e.g. you might be the lead PI or a co-PI), and list co-PIs for the new award.

None.

7. Were there any patents or copyrights as a result of this research? If so, please give the title of intellectual property, and a brief description of the property.

None.

8. Please list citations for any publications of research results from this project (can include outreach publications such as brochures or manuals, or research publications such as journal articles). Please also indicate if the publication was peer reviewed.

None.

9. Technology transfer or impact – please provide a brief description of any technology transfer and a summary of the impact on technology adoption or deployment.

None yet, but the planned workshop with Dr. Robert Hirsch to teach his Weighted Regressions on Discharge Time and Season analysis technique is scheduled for Nov. 1-2, 2018.

10. Please list any service or involvement in an organization or on an external advisory group that was a direct result of funding for this project (include group name, description of interactions and role/appointments and work performed).

PI Hall and Co-PI Paerl serve on the North Carolina Nutrient Criteria Development Plan's Scientific Advisory Committee to inform strategies for improving water quality on the Middle Cape Fear River, and eventually all of NC's surface waters. PI Hall has discussed the increasing nutrient load on the Cape Fear River that is largely due to point sources, and the fact that stemming eutrophication related water quality will likely require managing riverine nutrient concentrations rather than loads. PI-Hall also gave a presentation on water quality related statistics, contributed to a proposed new pH standard, and is currently contributing to the development of a proposal for a new chlorophyll a standard.

PI Hall joined the Cape Fear River Partners water quality committee and has contributed to the development of their five year (2018-2022) implementation plan.

To aid the Basinwide Planning Branch of the Division of Water Resources in preparation of the new Cape Fear River basinwide plan, PI-Hall provided a document highlighting the major trends in water quality along the Cape Fear River and trend analyses of point source loading of N, P, and ammonia broken down by sub-basin.

PI Hall mentored one undergraduate student, Sarah Hudak, in completing an independent study project on zooplankton grazing of cyanobacteria in Jordan Lake.

11. Have any students that graduated and were supported from funding from this project received professional placement? If so, please specify the economic sector in which they became employed.

None.

12. Is there anything else significant you'd like to share regarding your project?

the Lumbee River Basin post-Hurricane Matthew. Closing the data availability gap for basin residents, and helping enhance planning for future storms.

Nutrient Dynamics of the Lumbee River Basin post-Hurricane Matthew. Closing the data availability gap for basin residents, and helping enhance planning for future storms.

Basic Information

| | |
|---------------------------------|--|
| Title: | Nutrient Dynamics of the Lumbee River Basin post-Hurricane Matthew. Closing the data availability gap for basin residents, and helping enhance planning for future storms. |
| Project Number: | 2017NC216B |
| Start Date: | 7/15/2017 |
| End Date: | 2/28/2018 |
| Funding Source: | 104B |
| Congressional District: | NC-04 |
| Research Category: | Water Quality |
| Focus Categories: | Hydrology, Nutrients, Surface Water |
| Descriptors: | None |
| Principal Investigators: | Ryan E. Emanuel, Justine Neville |

Publications

There are no publications.

Nutrient Dynamics of the Lumbee River Basin post-Hurricane Matthew: Closing
the data availability gap for basin residents

WRI Project # 17-01-W

Progress Report
May 15, 2018

Prepared by:
Ryan Emanuel (faculty PI) and Justine Neville (student PI)

Project Overview

In October 2016, Hurricane Matthew brought unprecedented flooding to the Lumbee River basin in Southeastern North Carolina. Peak discharge at the USGS stream gage near Boardman, NC (Station Number 0234500) exceeded $1000 \text{ m}^3\text{s}^{-1}$, more than double the peak discharge following Hurricane Floyd, which had been the basin's worst flood in living memory (Figure 1). Regional flooding was widespread across the basin, especially southwest of downtown Lumberton, NC (Figure 2). Observations from the southwest side of Lumberton suggest that massive amounts of sediment were transported down the main stem of the Lumbee River before being deposited in neighborhoods close to or within the floodplain (Figure 3).

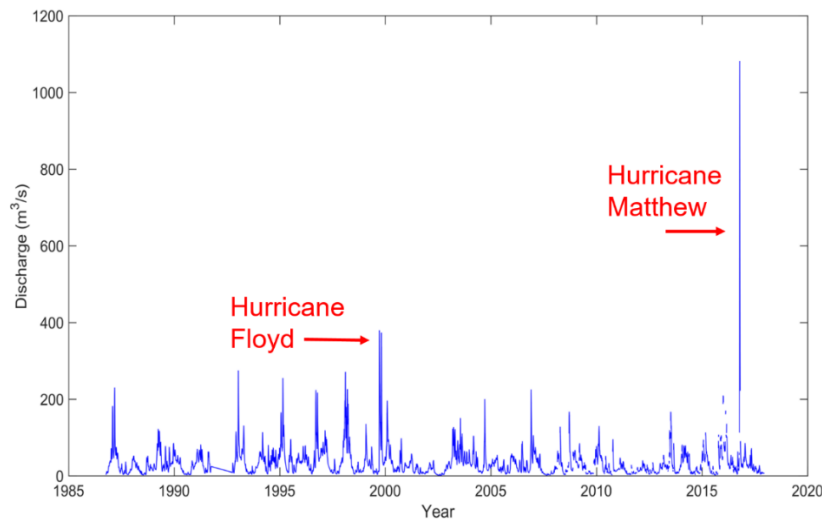


Figure 1: Hydrograph of Lumbee River (USGS Station 0234500) showing flood peaks following Hurricane Floyd (1999) and Hurricane Matthew (2016).

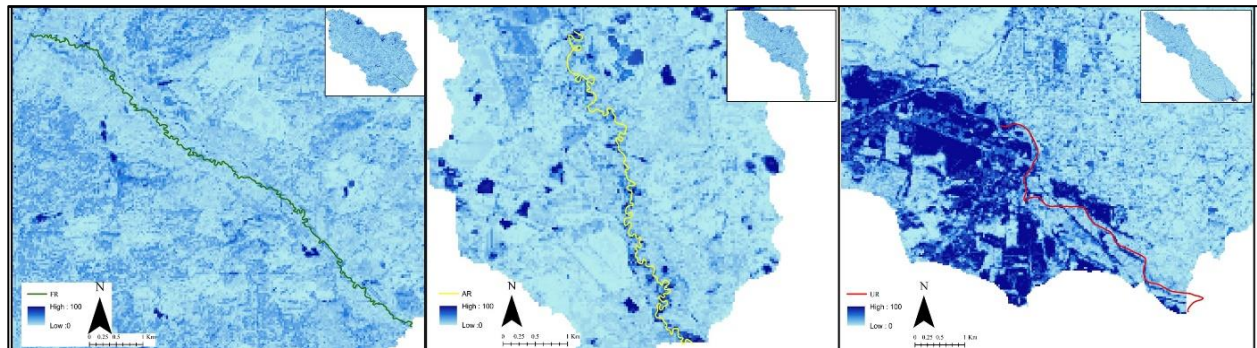


Figure 2: Estimates of flood extent following Hurricane Matthew using satellite imagery analyzed by Dr. Joshua Gray (NCSU).



Figure 3: Sediment deposited by Hurricane Matthew flooding in a neighborhood southwest of downtown Lumberton, NC.

The timing of Hurricane Matthew coincided with a year-long study of the spatial variability of dissolved nitrate (NO_3^-) in three reaches of the Lumbee River characterized by their proximal land use: forested, agricultural, and urban. Flooding caused by Hurricane Matthew provided a unique opportunity to study in-stream nitrogen processing before and after the flood. Potential sediment transport, addition of woody debris, temporary connectivity of nutrient sources, and other phenomena associated with the flood may have altered the river's capacity to either retain NO_3^- or to deliver this nutrient to downstream waters.

This work focused on three reaches that are part of an ongoing study of water quality in the Lumbee River basin (Figure 4). Synoptic NO_3^- samples collected along each reach were combined with estimates of streambed area and used to compute NO_3^- retention values for each reach and sampling date. Each reach was sampled 4-5 times before and after Hurricane Matthew, resulting in a distribution of NO_3^- retention values for each reach and date. The resulting dataset included a total of 28 distributions of NO_3^- retention from all three reaches, 14 in the months before Hurricane Matthew, and 14 in the months after the storm. We used these distributions in Wilcoxon Rank-Sum and 2-Sample Kolmogorov Smirnov tests to determine whether or not NO_3^- retention differed significantly for each reach before and after Hurricane Matthew.

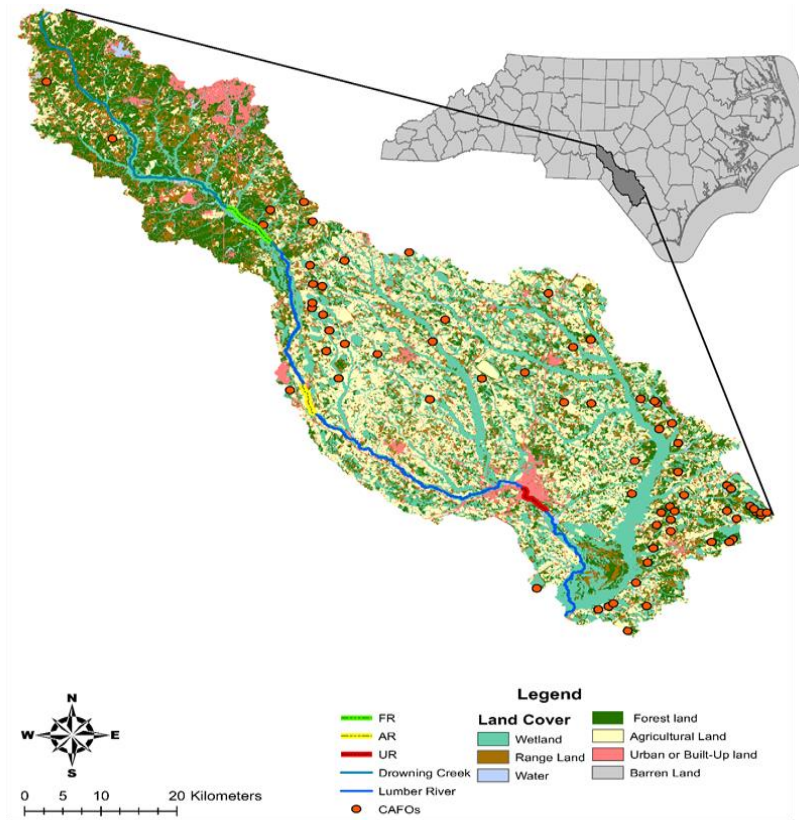


Figure 4: Land cover and study reaches within the Lumber River basin. Inset shows location of river basin within North Carolina.

Results

All of the stream reaches acted as both sources and sinks for NO_3^- on any given sampling date; however, all three reaches retained more stream NO_3^- prior to Hurricane Matthew than before the storm (Table 1). On sampling dates after Hurricane Matthew, all reaches became less efficient at retaining NO_3^- , but the rate of NO_3^- removal was greater. Moreover, the spatial variability in NO_3^- retention increased significantly following Hurricane Matthew (Figure 5). Whereas NO_3^- retention was fairly homogeneous prior to the hurricane, the range of sources and sinks along reaches increased substantially afterward.

Table 1: Nitrate (NO_3^-) retention statistics for study reaches

| | Pre HM | | | | Post HM | | | |
|--------------|------------------------|---------------------|-------------------|---------------------|------------------------|---------------------|-------------------|---------------------|
| | Mean Percent Retention | Interquartile Range | Mean Areal Uptake | Interquartile Range | Mean Percent Retention | Interquartile Range | Mean Areal Uptake | Interquartile Range |
| Forested | 0.2 | 2.99 | -45.86 | 202.44 | -2.84 | 6.70 | -2.84 | 106.06 |
| Agricultural | 0.32 | 4.43 | -169.91 | 442.08 | -3.14 | 8.22 | 26.38 | 162.26 |
| Urban | -0.24 | 4.89 | -13.01 | 720.19 | -0.65 | 7.80 | 12.00 | 134.63 |

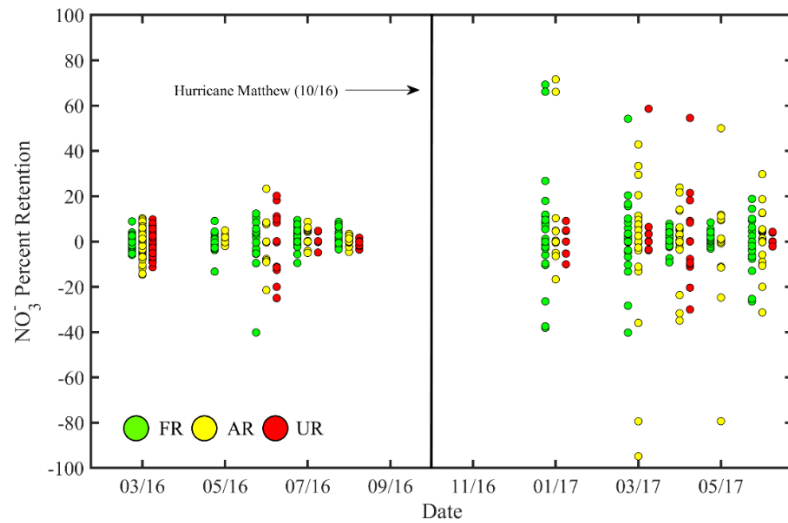


Figure 5: Percent NO₃- retention for forested (FR), agricultural (AR) and urban (UR) reaches before and after Hurricane Matthew.

We are continuing to interpret these results in an effort to understand implications for local environments and downstream waters, including estuaries that receive massive nutrient fluxes following storms such as Hurricane Matthew. The high spatial resolution of our dataset gives us a unique opportunity to study the variability of nitrogen processing within a reach, which has the potential to reveal new insight about how biogeochemical functions of streams are altered during major floods.

Products

To date, the project has allowed one NCSU Master's student (Justine Neville) to successfully complete and defend her thesis. The project provided direct funding to Neville and allowed her to travel to the 2017 American Geophysical Union Fall Meeting to present her work. It has also provided lab training for one undergraduate research assistant (Jalen Rose). Products to date include two poster presentations, led by Neville, at the AGU Fall Meeting and the 2018 NC Water Resources Research Institute Conference. The project has supported two scholarly manuscripts, which are currently in preparation.

Racial Disparities in Access to Clean Water in North Carolina: Communicating Health Risk to Private Well Owners

Basic Information

| | |
|---------------------------------|---|
| Title: | Racial Disparities in Access to Clean Water in North Carolina: Communicating Health Risk to Private Well Owners |
| Project Number: | 2017NC217B |
| Start Date: | 7/15/2017 |
| End Date: | 2/28/2018 |
| Funding Source: | 104B |
| Congressional District: | NC-04 |
| Research Category: | Social Sciences |
| Focus Categories: | Water Supply, Water Quantity, Education |
| Descriptors: | None |
| Principal Investigators: | Jacqueline MacDonald Gibson, Frank Stillo |

Publications

There are no publications.

Please answer questions as they apply to activities between
March 1, 2017 and February 28, 2018
Please return to Nicole_Wilkinson@ncsu.edu by Monday, May 14, 2018.

Specific Reporting Questions:

1. A. Please specify the number of each of the following who worked on this project:

B. Please also indicate in parentheses whether their field of study was engineering, physical sciences, biological sciences, natural resources/agricultural sciences, social sciences, or other (define other).

C. Please include the students' names.

D. Finally, please also indicate the number of faculty working on this project.

-Undergraduates: Sydney Lockhart, Public Health & Anthropology; Shirley Pu, Biological Sciences; Simran Khadka, Public Health
-Master's: Erica Wood, Public Health; Peter Kane, Public Health
-PhD: Frank Stillo, Public Health
-Faculty: Dr. Jackie MacDonald Gibson, Gillings School of Global Public Health
2. Did you or your students receive any awards or recognition for the research performed under this grant (example: a student received an honorable mention for a presentation delivered at a conference based on research results from this project)? If so, please specify the nature of the award (date, name of award, person receiving award, and organization/conference issuing the award).
-Not yet.
3. Please list any presentations, workshops, technology demonstrations, outreach events etc delivered based on research results from this project (include date, title, location of presentation and/or conference, and a brief description of session). If a workshop was held, please include number of attendees.
-Frank Stillo Presented results to Water Resources Research Institute on March 15th at NC State University. Title: *Community engagement to determine influences on well water testing: a mental models approach*
4. If student attendance at a conference was supported with funds from this project, please list the title of the conference and number of students who attended (note, this can be attendance only, and does not have to include giving a presentation, which would be covered under question 4).
5. Were any teaching assistantships given as a result of the award?

-No

6. Have you received any additional grants or funding as a supplement to this award, or for which this research served as the foundation? If so, please provide the title of the new grant/project/award, source of funding, amount of funding received, who the project was awarded to (e.g. you might be the lead PI or a co-PI), and list co-PIs for the new award.

-(1) U.S. EPA STAR Grant; "Water Infrastructure to Improve Childhood Health and Decrease Childhood Lead Exposure"; \$800,000; Lead PI, Jacqueline MacDonald Gibson; co-PIs: Keith Levine, John M. MacDonald, Philip J. Cook, Wandu Bruine de Bruin, Michael Fisher Institutions: University of North Carolina, Chapel Hill, NC (Gibson and Fisher); Research Triangle Institute, Durham, NC (Levine); University of Pennsylvania, Philadelphia, PA (MacDonald); Duke University, Durham, NC (Cook); University of Leeds, United Kingdom (Bruine de Bruin)

(2) NC Policy Collaboratory; "Lead and Emerging Contaminants in Private Wells in North Carolina – Risks and Solutions"; \$300,000; Lead PI Jacqueline MacDonald Gibson; Co-PIs: Detlef Knappe, NC State; Mei Sun, UNC Charlotte; Jamie DeWitt, ECU; Ralph Mead, UNC Wilmington; Lee Ferguson, Duke; Jeff Hughes, UNC CH

(3) NC Division of Health and Human Services: "Risk Communication to Private Well Owners"; \$34,317 ; Jacqueline MacDonald Gibson – Lead PI

7. Were there any patents or copyrights as a result of this research? If so, please give the title of intellectual property, and a brief description of the property.

-No

8. Please list citations for any publications of research results from this project (can include outreach publications such as brochures or manuals, or research publications such as journal articles). Please also indicate if the publication was peer reviewed.

-None to date, plan to submit to the Journal Risk Analysis in June.

9. Technology transfer or impact – please provide a brief description of any technology transfer and a summary of the impact on technology adoption or deployment.

-NA

10. Please list any service or involvement in an organization or on an external advisory group that was a direct result of funding for this project (include group name, description of interactions and role/appointments and work performed).

-Jackie was appointed to serve on the Science Advisory Board for the NC Department of Health and Human Services and NC Department of Environmental Quality to advise on issues of drinking water quality and water quality policies.

11. Have any students that graduated and were supported from funding from this project received professional placement? If so, please specify the economic sector in which they became employed.

-All students are still in school.

12. Is there anything else significant you'd like to share regarding your project?

- This work is leading to only the second randomized-controlled trial of a risk communication intervention for private well owners anywhere in the developed world.

Information Transfer Program Introduction

The Water Resources Research Institute (WRRI) is designed to provide water resources information to a range of stakeholders including private industry, academics, non-profit groups, and governmental entities. WRRI maintains a strong information transfer program by cooperating with various state agencies, municipalities, and professional organizations to sponsor conferences, workshops and other educational events, as well as seeking grants for relevant activities and publishing and distributing research results.

WRRI's signature training and outreach event, the WRRI Annual Conference, celebrated its 19th year in FY17. The WRRI Annual Conference continues to be the premier conference highlighting diverse topics in water research, management and policy in North Carolina. The event crosses multiple sectors, including academia, private consulting, local, state and federal government, non-profits and many others, and touches on the wide variety of disciplines that address water resources. The conference features oral and poster presentations, themed panel discussions, ample networking opportunities, and hands-on interactive sessions for more in-depth discussions and problem solving related to water resources. The 2017 conference brought an international perspective through the Nile Project that related to problem solving and collaboration here in our state. The Nile Project was presented in partnership with NC State LIVE, NC State University's performing arts program whose mission is to connect artists and audiences in a meaningful exploration of the diverse cultures and issues that define our communities and world. Feedback from participants is consistently positive, with many noting that this is their preferred and priority conference to attend each year, and that they gain more from WRRI that applies to their work than from any other conference they attend.

WRRI continues to administer the NC Urban Water Consortium (UWC) and the UWC-Stormwater Group (SWG), which comprise drinking/wastewater utilities and municipal stormwater programs, respectively. WRRI plays an active role in developing agendas for quarterly meetings for each group (a total of 8 held during this reporting period) that highlight emerging priority research projects in the state, exploring topics of concern for each group, and pursuing opportunities to educate and engage group members to better support their management activities and needs.

In FY 17, WRRI expanded the professional development credits it is able to offer to event participants. It continues to sponsor continuing education credits by the NC Board of Examiners of Engineers and Surveyors as an Approved Sponsor of Continuing Professional Competency activity for Professional Engineers and Surveyors licensed by the State of North Carolina. In addition, WRRI submits information for approval to the N.C. Board of Landscape Architects to offer contact hours to landscape architects. This allows WRRI to offer Professional Development Hours (PDHs) to engineers and surveyors, and Continuing Education Units (CEUs) to landscape architects for attendance at the WRRI Annual Conference and other workshops, seminars and forums that WRRI sponsors. This year, WRRI was also approved to offer professional development credits for licensed geologists and for soil scientists, furthering both the value of our program to stakeholders and confirmation of the quality of WRRI programming.

WRRI continues to expand its activities under the umbrella of the Center of Excellence for Watershed Management (CEWM). Through the CEWM, WRRI's Sustainable Waters and Communities Coordinator helps communities identify local opportunities and implement sustainable practices for managing their waters. Community leadership and participation in watershed efforts are critical to protecting waters, and the CEWM provides services and support for these efforts. The CEWM aids communities by supporting the NC Watershed Stewardship Network (NCWSN), providing tools and training opportunities, and coordinating local watershed specific projects. During this reporting period, 1711 square feet of green infrastructure (rain gardens, bioretention and cisterns) were installed in communities across central North Carolina, treating stormwater runoff from a total of 19,400 square feet of impervious surface. An additional \$260,258 of external grants were received to continue watershed stewardship activities.

Information Transfer Program Introduction

Through 22 education and training events (not counting many additional presentations and stakeholder meetings), WRI reached 1105 adult participants and 147 youth participants.

WRRRI Informtaion Transfer Program

Basic Information

| | |
|---------------------------------|------------------------------------|
| Title: | WRRRI Informtaion Transfer Program |
| Project Number: | 2017NC213B |
| Start Date: | 3/1/2017 |
| End Date: | 2/28/2018 |
| Funding Source: | 104B |
| Congressional District: | NC-004 |
| Research Category: | Not Applicable |
| Focus Categories: | None, None, None |
| Descriptors: | None |
| Principal Investigators: | Nicole Wilkinson |

Publications

1. Mallin, M.A., M.R. McIver and N. Iraola. 2018. Environmental Quality of Wilmington and New Hanover County Watersheds, 2017. UNCW-CMS Report 18-01, Center for Marine Science, University of North Carolina at Wilmington, Wilmington, N.C. (Non-peer reviewed technical report)
2. Wiltse D., Schnetzer A., Green J., Vander Borgh M. and Fensin E. 2018. Algal Blooms and Cyanotoxins in Jordan Lake, North Carolina Toxins 10(2), 92
3. de los Reyes, Francis and Tarek Aziz. 2017. Improving startup and operation of anaerobic co-digestion of grease interceptor waste. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-468, 29 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35249>
4. Call, Douglas, Francis de los Reyes and Qiwen Cheng. 2017. Improving the Anaerobic Treatment of Sludges and High-Strength Wastewaters through Addition of Electrically-Conductive Particles. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-469, 28 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35250>
5. Sobsey, Mark et al. 2017. Microbial Quality and Risk Assessment of Type 2 NC Reclaimed Water for Non-Potable and Potable Reuse. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-467, 133 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35248>
6. Yingling, Yaroslava G. and James S. Peerless. 2018. Computational development of composite packed-bed nanofiltration for high-throughput desalination. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-472, 13 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35253>

FY 2017 NC WRRRI Information Transfer Program Report

WRRRI-SPONSORED WORKSHOPS, FORUMS AND SEMINARS

Table 1 records the educational and training events WRRRI sponsored during the project year, along with a description of each and the number of attendees. Through these events and programs, WRRRI engaged a documented 1105 adult participants and 147 youth participants, though many events targeted additional unquantified audiences through webinars. Additional stakeholders were reached through a number of meetings, focus groups, and other gatherings that are not captured below in the official list of training events.

Table 1: Structured education, outreach and training events for FY17.

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|-------------------|---|-------------|--------------------|--------------------|--|
| 03/15-03/16, 2017 | 19th WRRRI Annual Conference | Raleigh, NC | 312 | | For 19 years, the WRRRI Annual Conference has been the premier conference highlighting diverse topics in water research, management and policy in North Carolina. The conference featured oral and poster presentations, themed panel discussions, ample networking opportunities, and hands-on interactive sessions for more in-depth discussions and problem solving related to water resources. The 2017 conference brought an international perspective through the Nile Project that related to problem solving and collaboration here in our state. The Nile Project was presented in partnership with NC State LIVE, NCSU's performing arts program whose mission it is to connect artists and audiences in a meaningful exploration of the diverse cultures and issues that define our communities and world. |
| 4/22/2017 | Kingswood Elementary STEM Expo display and engagement | Cary, NC | 20 | 20 | At the school's annual STEM Expo, WRRRI and partner Natural Learning Initiative, NCSU College of Design, hosted an interactive display with educational materials about rain gardens and rainwater harvesting, and graduate student designs for a rain garden and cistern. Parents and students provided feedback on designs, while younger children played with a watershed model made of kinetic sand and marbles. |
| 4/23/2017 | St. Ambrose Rain Garden Installation and Planting | Raleigh, NC | 16 | 6 | As part of the Walnut Creek Wetland Community Partnership, in which WRRRI participates as part of its efforts as a Center of Excellence for Watershed Management, this event engaged community members and parishioners in the planting of a rain garden at St. Ambrose church in the Walnut Creek watershed. 515 square feet of rain garden were installed. |
| 5/4/2017 | Greater Triangle Stewardship Development Awards Event | Raleigh, NC | 50 | | Four land development projects were honored during the seventh annual awards ceremony for the Greater Triangle Stewardship Development Awards Program (GTSDA). These awards recognize development projects in the Triangle area that go above and beyond state and local requirements to incorporate innovative environmental protections and provide a model for green development practices. This year's winners included Chatham Park Medical Office Building #2 in Chatham County, Wooten Meadow Park Master Plan in Raleigh, and NC State's Talley Student Union in Raleigh, with the highest honors going to the Hungry Neck Residence in Raleigh. The 2017 awards marked the first time applications were opened to single family homes. WRRRI is represented on the GTSDA Board and assisted with the planning of this event as well as the solicitation and selection of winners, and was also an official event sponsor. |

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|-----------------|--|----------------|--------------------|--------------------|--|
| 5/31/2017 | Beechtree Residential Rain Garden Planting | | 7 | 5 | Volunteers, including high school students from Town of Cary's Teen Council, learned about rain gardens and installed plants in four residential rain gardens. |
| 6/1/2017 | Kingswood Elementary School classes Outdoor Data Exploration | Cary, NC | 4 | 70 | Three fourth grade and one fifth grade classes and their teachers collected site data pre-construction to establish conditions prior to a rain garden installation, using Wisconsin Arboretum materials. Randy Senzig, of the Center for Human Earth Restoration, led the students. |
| 8/6/2017 | Think Blue - NC Watershed Awareness Outreach | Durham, NC | 60 | | Think Blue was an event that brought together organizations and scientists from around the Durham and North Carolina regions who are along the spectrum of environmental sustainability and public health. Specifically, each organization is committed to clean and healthy water for both human and environmental health. Fifteen groups participated by tabling the event and provided games, activities, and other types of materials to engage with members of the local community. This was a festival-style event where people were encouraged to come by and interact with the organizations and researchers at the booths. |
| 9/14-9/15, 2017 | 2017 Confluence Conference: Protecting our water resources, are you up to the challenge? | Charleston, SC | 129 | | For 19 years, NC, SC and GA have partnered to host the Confluence Conference, a tri-state gathering of water professionals that focuses on sharing lessons learned and building collaborations across these three states who share river basins, similar hydrogeographic regions, and similar socioeconomic characteristics. Unlike larger meetings that focus on the broader Southeast region, Confluence has become an annual home to many water professionals, particularly those working at high levels in local water utilities and representing water resources at their respective state agencies and professional organizations. The theme for 2017 was "Protecting our Water Resources: Are you up to the Challenges?" The concept of "challenges" was highlighted by the passing of Hurricane Irma, which flooded Charleston the day before the conference began. We retained the majority of our attendees, and it provided great, real-time fodder for discussions about water-related challenges, including emergency management, public safety, communications, and risk. The conference was lucky to have the mayor of Flint, Michigan, Ms. Karen Weaver, as the keynote speaker. |

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|------------|---|------------------------------|--------------------|--------------------|--|
| 9/25/2017 | NCWRA Forum & Webinar, "Emerging Contaminants: Gen X and the Cape Fear River" | McKimmon Center, Raleigh, NC | 84 | | Ms. Sheila Holman, NC DEQ, shared insight on the state's work related to emerging contaminants in our water systems and provided an update on results and strategies related to the state's investigation into Gen X discharge from the Chemours, Fayetteville facility. |
| 9/27/2017 | NC Watershed Stewardship Network: Sustainably Funding Your Watershed Efforts | Newton, NC | 39 | | NC Watershed Stewardship Network presented a new daylong workshop this fall, "Sustainably Funding your Watershed Efforts" to learn about and discuss strategies for obtaining funds and leveraging resources to support watershed protection and restoration efforts. Participants from non-profits and informal watershed groups, municipalities, and conservation agencies were encouraged to attend along with others from their watersheds. Participants learned about public and private sources of funding through "lightning round" presentations by funding organizations, heard local watershed groups' successful strategies, learned grant-writing tips, and considered how to apply strategies in their own watershed efforts. |
| 10/12/2017 | Kingswood Elementary School Rain Garden Planting | Cary, NC | 5 | 19 | A 4th grade class kicked off the planting of a large school rain garden with Randy Senzig and staff from the non-profit Center for Human and Earth Restoration. |
| 10/14/2017 | Kingswood Elementary School Volunteer Rain Garden Planting | Cary, NC | 9 | 3 | School staff, parents and students participated in planting the rain garden as part of STEM Saturday events. |
| 10/19/2017 | Neighborhood Ecology Corps Green Infrastructure Workshop | Raleigh, NC | | 14 | Middle and high school students learned about watershed science and green infrastructure at a hands-on workshop, in partnership with Center for Human and Earth Restoration. |

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|------------|--|-------------------|--------------------|--------------------|--|
| 10/23/2017 | NC Watershed Stewardship Network: Sustainably Funding Your Watershed Efforts | New Bern, NC | 33 | | NC Watershed Stewardship Network presented a new daylong workshop this fall, "Sustainably Funding your Watershed Efforts" to learn about and discuss strategies for obtaining funds and leveraging resources to support watershed protection and restoration efforts. Participants from non-profits and informal watershed groups, municipalities, and conservation agencies were encouraged to attend along with others from their watersheds. Participants learned about public and private sources of funding through "lightning round" presentations by funding organizations, heard local watershed groups' successful strategies, learned grant-writing tips, and considered how to apply strategies in their own watershed efforts. |
| 10/26/2017 | Kingswood Elementary School Volunteer Rain Garden Planting | Cary, NC | | 10 | Volunteers from WRRI and NC Sea Grant completed planting the school rain garden. |
| 11/2/2017 | TMDL Alternatives: Category 4B Success Stories and Other Options | Winston-Salem, NC | 60 | | The NC Water Resources Association and NC Association of Environmental Professionals co-hosted this workshop to focus on alternatives for addressing impaired waters without full TMDL development and implementation and highlighted case studies where such alternatives have been successfully implemented. Speakers represented EPA Region 4, NC Department of Environmental Quality and NC Department of Transportation. |
| 11/9/2017 | NC Watershed Stewardship Network: Sustainably Funding Your Watershed Efforts | Fayetteville, NC | 30 | | NC Watershed Stewardship Network presented a new daylong workshop this fall, "Sustainably Funding your Watershed Efforts" to learn about and discuss strategies for obtaining funds and leveraging resources to support watershed protection and restoration efforts. Participants from non-profits and informal watershed groups, municipalities, and conservation agencies were encouraged to attend along with others from their watersheds. Participants learned about public and private sources of funding through "lightning round" presentations by funding organizations, heard local watershed groups' successful strategies, learned grant-writing tips, and considered how to apply strategies in their own watershed efforts. |

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|------------|--|-------------|--------------------|--------------------|---|
| 11/14/2017 | Are You Ready For the Big One: Lessons Learned from Hurricane Matthew | Raleigh, NC | 64 | | This special session was convened by the NC American Water Works Association - Water Environment Association (NC AWWA-WEA) Water Resources Committee for the NC AWWA's annual state conference. Panelists from the National Oceanographic and Atmospheric Administration, US Geological Survey, three public water utilities, NC's State Dam Safety Program, and Innovative Emergency Management, Inc all provided unique perspectives about the challenges and lessons learned from Hurricane Matthew. This hurricane struck NC in October 2016 and produced record rainfall and flooding events, leaving communities displaced even today. Attendees from water utilities, local governments, and private consulting firms learned valuable lessons about communication, early warning systems, networks and crisis management. |
| 12/4/2017 | NCWRA Forum & Webinar "What's New in NC's Stormwater Program?" | Raleigh, NC | 90 | | In 2017, North Carolina's State Stormwater Program updated its design standards for stormwater control measures. Designers now have much more flexibility to treat stormwater in a more cost-effective manner that can enhance developments. Annette Lucas, stormwater program supervisor at the NC Department of Environmental Quality's Division of Energy, Mineral and Land Resources, shared some highlights of these updates and her ideas about how these changes can be put into practice. |
| 12/11/2017 | Kingswood Elementary School Boulder Planting | Cary, NC | 6 | | Community members and NCSU graduate students installed boulders in the school's new rain garden to provide walking steps and seating for interaction. |
| 2/5/2018 | NCWRA Forum & Webinar "Wake County Efforts to Protect Private Well Users from Contamination" | Raleigh, NC | 80 | | Private wells are an important component of the water resource infrastructure in Wake County, where nearly 15 percent of residents rely on private wells, and throughout North Carolina, where private wells serve roughly 25 percent of residents. Evan Kane, groundwater manager with Wake County Environmental Services, shared information on contamination risks facing private well users in Wake County and statewide and approaches Wake County has taken to address these risks in its permitting, outreach, and technical assistance programs. |

| Event Date | Event Title | Location | Adult Participants | Youth Participants | Event Description |
|------------|--------------------------------|-------------|--------------------|--------------------|---|
| 2/19/2018 | Effective Poster Presentations | Raleigh, NC | 7 | | Designed for students, faculty and professionals, this workshop emphasized effective visualization, design and communication of research. The workshop focused primarily on poster presentations but content was applicable to both poster and oral presentations at conferences. Participants submitted draft conference posters (several of which were to be presented at WRRRI's annual conference), and the participants and 4 workshop leaders reviewed it as a group during the workshop with consideration of design elements discussed in the first part of the workshop. |

PUBLICATIONS

8 external publications (7 of which were peer-reviewed) resulted from WRRRI-funded projects this period (including those funded with non-USGS funds).

- Blum, P.W. et al. 2017. Methylmercury and methane production potentials in North Carolina Piedmont stream sediments. *Biogeochemistry*, 137(1-2), pp181-195
<https://doi.org/10.1007/s10533-017-0408-8>
- Burtchett, J.M., Mallin, M.A. and Cahoon, L.B. 2017. Micro-zooplankton grazing as a means of fecal bacteria removal in stormwater BMPs. *Water Science & Technology*, 75(11), pp 2702-2715
- Hounshell, A.G. et al. 2017. Stimulation of Phytoplankton Production by Anthropogenic Dissolved Organic Nitrogen in a Coastal Plain Estuary. *Environ. Sci. Technol.* 2017 51 (22), 13104-13112. DOI: 10.1021/acs.est.7b03538
- Mallin, M.A., M.R. McIver and N. Iraola. 2018. Environmental Quality of Wilmington and New Hanover County Watersheds, 2017. UNCW-CMS Report 18-01, Center for Marine Science, University of North Carolina at Wilmington, Wilmington, N.C. (Non-peer reviewed technical report)
- Messer, T.M., M.R. Burchell, and F. Birgand. 2017. Nitrate removal potential of restored wetlands loaded with agricultural drainage water: A mesocosm scale experimental approach. *Ecological Engineering*. 106:541-554.
- Messer, T.M., M.R. Burchell, and F. Birgand. 2017. Comparison of four nitrate removal kinetic models in two distinct wetland restoration mesocosm systems. *Water*. 9(7) 517
[doi:10.3390/w9070517](https://doi.org/10.3390/w9070517)
- Messer, T.M., M.R. Burchell, J.K. Bohlke, and C. Tobias. 2017. Tracking the fate of nitrate through pulse-flow wetlands: A mesocosm scale 15N enrichment tracer study. *Ecological Engineering*. 106:597-608.
- Wiltsie D., Schnetzer A., Green J., Vander Borgh M. and Fensin E. 2018. Algal Blooms and Cyanotoxins in Jordan Lake, North Carolina *Toxins* 10(2), 92

WRRRI Published 6 internal technical reports during this reporting period.

- Bowen, James and Noyes Harrigan. 2017. Comparing the impact of organic versus inorganic nitrogen loading to the Neuse River Estuary with a mechanistic eutrophication model. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-470, 119 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35251>
- de los Reyes, Francis and Tarek Aziz. 2017. Improving startup and operation of anaerobic co-digestion of grease interceptor waste. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRRI-468, 29 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35249>

- Call, Douglas, Francis de los Reyes and Qiwen Cheng. 2017. Improving the Anaerobic Treatment of Sludges and High-Strength Wastewaters through Addition of Electrically-Conductive Particles. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRI-469, 28 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35250>
- Christenson, Elizabeth and Jill Stewart. 2018. Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent *E. coli* and host-specific markers in watersheds with and without swine CAFOs. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRI-471, 15 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35252>
- Sobsey, Mark et al. 2017. Microbial Quality and Risk Assessment of Type 2 NC Reclaimed Water for Non-Potable and Potable Reuse. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRI-467, 133 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35248>
- Yingling, Yaroslava G. and James S. Peerless. 2018. Computational development of composite packed-bed nanofiltration for high-throughput desalination. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRI-472, 13 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35253>

WRRI ELECTRONIC LISTS

WRRI maintains the following electronic mail lists (listservs) for information transfer purposes, which reach a combined total of almost 2000 people statewide:

- Water-Research list — informs water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.;
- WRRI-News list - informs researchers, local governments, municipalities, interest groups etc. about calls for papers, grants, upcoming conferences and events, etc.;
- NCWRA-info list - provides information of the North Carolina Water Resources Association sponsored events;
- Sediments list - used to disseminate erosion and sedimentation control information in North Carolina;
- Watershed Stewardship Network (WSN) list – provides watershed professionals, volunteers and stakeholders throughout the state with a mechanism to contact, network, and learn from each other as well as to learn about the WSN and its offerings;
- Urban Water Consortium (UWC) list for Urban Water Consortium member communications;
- and UWC-Stormwater Group list for the UWC Stormwater Group member communications.

NC URBAN WATER CONSORTIUM

WRRI administers the NC Urban Water Consortium (UWC) and meets with the members quarterly. The consortium was established in 1985 by the Institute, in cooperation with several of North Carolina's larger cities to provide a program of research and development, and technology transfer on water problems that urban areas share. Through this partnership, WRRI and the State of North Carolina help individual facilities and regions solve problems related to local environmental or regulatory circumstances. Participants support the program through annual dues and enhancement funds and

guide the program through representation on an advisory board, selection of research topics, participation in design of requests for proposals, and review of proposals. There are 12 member cities/special districts in North Carolina, and members hosted four quarterly meetings throughout the state in FY17.

NC URBAN WATER CONSORTIUM - STORMWATER GROUP

In 1998, several members of the NC UWC partnership formed a special group to sponsor research and technology transfer on issues related to urban stormwater and management. The Urban Water Consortium (UWC) Stormwater Group is administered by WRRRI. Participants support the program through annual dues and enhancement funds. They guide the program through selective representation on the WRRRI advisory board, determining stormwater-related research priorities, participation in the design of requests for proposals and review of proposals submitted to WRRRI directly or to the SWG. Four meetings were hosted by rotating SWG members throughout the state during the reporting year.

USGS Summer Intern Program

None.

| Student Support | | | | | |
|------------------------|-------------------------------|-------------------------------|-----------------------------|----------------------------|--------------|
| Category | Section 104 Base Grant | Section 104 NCGP Award | NIWR-USGS Internship | Supplemental Awards | Total |
| Undergraduate | 9 | 1 | 0 | 10 | 20 |
| Masters | 6 | 0 | 0 | 8 | 14 |
| Ph.D. | 5 | 1 | 0 | 10 | 16 |
| Post-Doc. | 0 | 0 | 0 | 0 | 0 |
| Total | 20 | 2 | 0 | 28 | 50 |

Notable Awards and Achievements

WRRI Staff Professional Development Leads to Opportunities for Educators WRRI program coordinator Anna Martin is pursuing her North Carolina Environmental Education certification. This program encourages professional development in environmental education and acknowledges educators committed to environmental stewardship, which Ms. Martin will bring to WRRI and stakeholders. This program establishes standards for professional excellence in environmental education for formal and non-formal educators. As part of this effort, Ms. Martin completed a course “Environmental Education in Practice” which also required a field placement assignment. As a natural resource major with experience working for the NC Water Resources Institute and NC Sea Grant, Ms. Martin chose to work with the NC Project WET (Water Education for Teachers) program coordinator. Project WET workshops, geared toward K-12 teachers and non-formal educators, emphasize a water literacy framework covering seven essential Principles of Water Science. The state coordinator had not yet presented any workshops on the newly published (March 2017) Getting Little Feet Wet (GLFW) curriculum, so she and Ms. Martin developed a workshop template based on this guide to use in future trainings across North Carolina. They delivered this 3-hour workshop with a total of seven early childhood educators participating offering three hours of Criteria III EE credit. In order to maximize the “train the trainer” mentality of this class, they presented the new guide in such a way that the attendees worked to “Become the Experts” at each activity. This was achieved by providing the needed resources for 8 of the 11 lessons in the guide and having the teachers work in pairs to present “mini-lessons” during the workshop detailing the objectives, methods of teaching to a pre-K audience, vocabulary learned, curriculum alignments and additional resources needed or modifications to be made while teaching. By providing early childhood educators with this hands-on experience and direct exposure to the lessons, they can quickly implement the GLFW curriculum into their formal teaching plans.

WRRI Grows Commitment to Equity and Diversity Across its Programs NC State University, home to WRRI, is one of 80 universities to receive a 2017 Higher Education Excellence in Diversity (HEED) award from “INSIGHT Into Diversity” magazine. The HEED award recognizes universities whose diversity and inclusion efforts show a broad understanding of diversity, including gender, race, ethnicity, military service, disabilities, membership in the LGBT community and more.

WRRI staff have become increasingly proactive in finding ways to become more inclusive programs, to recognizing and removing implicit biases, and to creating more opportunities for stakeholders who previously have been underserved by and underrepresented in our programs.

Of particular note in this area is that Christy Perrin, WRRI’s sustainable waters and communities coordinator, completed NCSU’s Equal Opportunity Institute, a year-long certificate course where Perrin increased her knowledge of equal opportunity issues in the workplace, and developed skills necessary to cope with diverse working and learning environments.

“We don’t know what we don’t know,” Perrin says, referring to a key lesson. “Experiences of discrimination, micro-aggressions, exclusion and institutional barriers can be invisible to those who haven’t experienced them.”

By learning about federal and university equal opportunity protections, Perrin developed skills and confidence for forming new partnerships. “For me, the most powerful part of the institute came with hearing stories from — and conversing with — fellow participants.”

Through WRRI, Perrin leads initiatives that involve multiple sectors and residents in planning, studying, protecting and restoring water resources in a sustainable manner. Her special interests include helping organizations enhance community development, particularly in historically underserved areas.

“I feel encouraged that NC State offers the program and that so many participate. I also appreciate the administration of WRRI and NC Sea Grant providing unwavering support for our team’s efforts to improve equity, diversity and inclusion in our work,” Perrin adds.

WRRI’s Excellence in Watershed Management Efforts Leads to Additional Funding In FY17, WRRI continued its commitment to the NC Watershed Stewardship Network (WSN), which was formed through a collaboration in which WRRI was highly active and engaged, with continued funding for its Sustainable Waters and Communities Coordinator to serve part-time as co-coordinator of the network. The Sustainable Waters and Communities Coordinator continues to manage community watershed restoration efforts funded and supplemented by EPA 319 grants and cost-sharing contributed by partnering organizations. These include the Black Creek Watershed Association in the Neuse River Basin and the town of Cary; and the Walnut Creek Wetland Community Partnership in southeast Raleigh. These projects involve engaging local municipal and citizen partners in education, installing stormwater control measures to reduce urban runoff, and monitoring impacts. Through the partnerships, expertise and momentum gained through these efforts, three external grants totaling \$260,258 were received to continue these efforts. They are:

- Low Impact Development Hotel in the Black Creek Watershed, received from US EPA Clean Water Act Section 319 for \$210,258.
- Green Infrastructure Education and Stream Monitoring, received from American Rivers, for \$10,000
- Urban Parks with Purpose Subgrant, received from the NC Conservation Fund (prime sponsor is the JPB Foundation), for \$40,000

Student Awards, Achievements and Impacts WRRI supported 22 students through the FY17 projects that were supported by USGS. In total, 50 students were supported across all projects that were active with WRRI during this reporting period.

Justine Neville, a graduate researcher on project 2017NC216B under lead PI Ryan Emanuel at NC State University, successfully completed her MS and enrolled in NCSU’s PhD program within Dr. Emanuel’s department. She is working on two manuscripts from her master’s and will acknowledge the WRRI award in those publications.

While the projects below were awarded in the prior fiscal year, these accomplishments occurred during the current reporting period.

Noyes Harrigan received several awards for his poster presentations that were based upon the work from a USGS project under lead PI James Bowen, UNC-Charlotte. In the previous year of the project, Noyes won a WRRI travel scholarship and the NC AWWA student poster competition award. This year, Noyes won the best student poster competition at the NC WRRI Annual Conference March 15-16, 2017. In June 2017, after earlier winning the regional AWWA student poster competition with his entry “Three for the Price of Two? A comparison of circulation in the Neuse River Estuary predicted by a two and three-dimensional model,” Noyes competed in the national competition that was held at the AWWA Annual Conference in Philadelphia, PA. Noyes also received a teaching assistantship from the Department of Civil and Environmental Engineering at UNC Charlotte as a result of this award.

Kirsten Studer, a PhD student working on a project under lead PI Howard Weinberg, UNC-Chapel Hill, received a Certificate of Merit Award for her oral presentation at the American Chemical Society National Meeting & Exposition in Washington D.C. in August 2017.

WRRI-Sponsored Research Leads to Additional Funding Awards WRRI’s FY17 researchers that were supported with USGS funds secured an additional \$1,279,674 in grant funding for continuation and expansion of the research topics explored through the WRRI-funded projects. They are detailed below.

PI Michael Burchell, NC State University, obtained \$7700 from the North Carolina Coastal Federation for a project entitled: “Restored wetlands to improve water quality in agricultural watersheds – a pilot-scale study” based on his WRRI project 2016NC209B.

PI Avner Vengosh, Duke University, received a grant for \$137,657 from the National Science Foundation Exploratory Research (EAGER) program entitled: The occurrence and distribution of hexavalent chromium and other contaminants in groundwater from aquifers of the Eastern United States. (EAR-1733637). This was based off of his WRRI project 2017NC211B.

Jacqueline McDonald Gibson, University of North Carolina at Chapel Hill, received three additional grants as lead PI for which WRRI project 2017NC217B served as the foundation. The grants include:

- U.S. EPA STAR Grant “Water Infrastructure to Improve Childhood Health and Decrease Childhood Lead Exposure” for \$800,000
- NC Policy Collaboratory Grant “Lead and Emerging Contaminants in Private Wells in North Carolina – Risks and Solutions” for \$300,000
- NC Division of Health and Human Services Grant “Risk Communication to Private Well Owners” for \$34,317

In total, researchers with active projects during this reporting period who were funded through WRRI (including from USGS funds, state funds, and other consortia funds) successfully secured a total of \$1,369,625 in additional funding outside of WRRI for which their WRRI-supported project served as the foundation. This additional funding does not include the additional state match and private consortia funds that WRRI uses to match USGS funds and award additional research dollars.

Service on Boards and Committees WRRI team members are actively engaged in local, state and national board and committee activities where they bring expertise and perspective to efforts to address water issues. WRRI is represented on the following:

- Universities Council on Water Resources (UCOWR) - Board of Directors
- National Institutes for Water Resources – Board of Directors
- NC Water Resources Association Board of Directors
- Greater Triangle Stewardship Development Association - Board of Directors
- NC Sedimentation Control Commission - Chair
- NC Nutrient Criteria Implementation Committee
- NC American Water Works Association - Water Resources Committee
- NC Coastal Reserve/NC National Estuarine Research Reserve – Education Advisory Committee
- NC Coastal Federation – Oyster Steering Committee
- NC Sentinel Site Cooperative – Core Management Team
- Albemarle-Pamlico National Estuary Partnership – Water Resources Committee; Decision Support Tools Action Team Committee; and Scientific Technical Advisory Committee
- NC State University Health and Wellness Committee – Wellness Champion Team
- Walnut Creek Wetland Park Master Plan - Citizen Planning Committee

Publications from Prior Years

1. 2015NC192B ("Legacy impacts of coal combustion residues on freshwater ecosystems in North Carolina") - Articles in Refereed Scientific Journals - Brandt, J.E.; Bernhardt, E.S.; Dwyer, G.S.; and Di Giulio, R.T.; 2017. Selenium ecotoxicology in freshwater lakes receiving coal combustion residual effluents: A North Carolina example. *Environ Sci & Technol.* Article ASAP DOI: 10.1021/acs.est.6b05353
2. 2015NC193B ("Coal ash constituents at the base of aquatic food webs: Processes affecting bioaccumulation and trophic transfer of arsenic") - Articles in Refereed Scientific Journals - Lopez, A.R., D.H. Funk and D.B. Buchwalter. 2017. Arsenic (V) bioconcentration kinetics in freshwater macroinvertebrates and periphyton is influenced by pH. *Environmental Pollution.* 224:82-88.
3. 2015NC191B ("Heavy metal analysis, gene proxies, and stable isotope tracers of coal ash contamination in the Dan River food web") - Articles in Refereed Scientific Journals - Blum, P.W. et al. 2017. Methylmercury and methane production potentials in North Carolina Piedmont stream sediments. *Biogeochemistry*, 137(1-2), pp181-195 <https://doi.org/10.1007/s10533-017-0408-8>
4. 2016NC199B ("Particle-bound Nutrients in Stormflow: A New Approach for Monitoring and Predicting N and P Transport and Fate in Watersheds of the NC Piedmont") - Articles in Refereed Scientific Journals - Hounshell, A.G. et al. 2017. Stimulation of Phytoplankton Production by Anthropogenic Dissolved Organic Nitrogen in a Coastal Plain Estuary. *Environ. Sci. Technol.* 2017 51 (22), 13104-13112. DOI: 10.1021/acs.est.7b03538
5. 2014NC186B ("Quantification of Fecal Bacteria Removal by Micro-zooplankton Grazing in Stormwater BMPs") - Articles in Refereed Scientific Journals - Burtchett, J.M., Mallin, M.A. and Cahoon, L.B. 2017. Micro-zooplankton grazing as a means of fecal bacteria removal in stormwater BMPs. *Water Science & Technology*, 75(11), pp 2702-2715
6. 2016NC201B ("Comparing the Impact of Organic vs. Inorganic Nitrogen Loading to the Neuse Estuary with a Mechanistic Eutrophication Model") - Water Resources Research Institute Reports - Bowen, James and Noyes Harrigan. 2017. Comparing the impact of organic versus inorganic nitrogen loading to the Neuse River Estuary with a mechanistic eutrophication model. NC Water Resources Research Technical Report. North Carolina State University, Raleigh, NC. Report No. UNC-WRRI-470, 119 pp. URI: <http://www.lib.ncsu.edu/resolver/1840.20/35251>